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Phytoplankton Growth Rates in the Ross Sea, Antarctica

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Phytoplankton Growth Rates in the Ross Sea, Antarctica

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A Thesis

Presented to

The Faculty of the School of Marine Science

The College of William and Mary in Virginia

In Partial Fulfillment

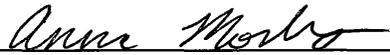of the Requirements for the Degree of

Master of Science

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by

Anna Ford Mosby

2013

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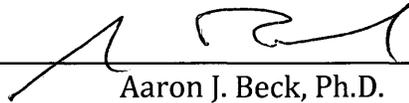
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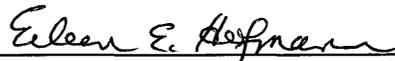Anna F. Mosby

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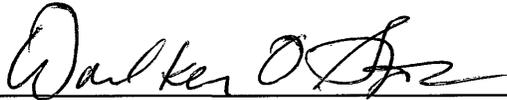
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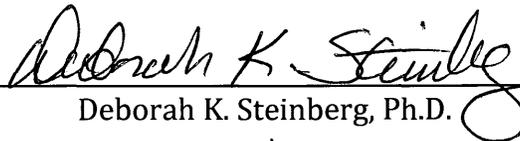Committee Chairman/Advisor

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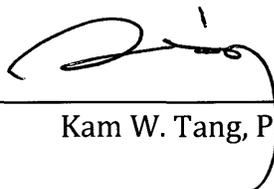Eileen E. Hofmann, Ph.D.

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ABSTRACT

The Ross Sea is a highly productive region of the Southern Ocean characterized by spatially variable distribution of phytoplankton, primarily *Phaeocystis antarctica*, but phytoplankton growth rates in the region have not been thoroughly investigated. Variability in growth rates was investigated from January to February 2012 on a cruise to the Ross Sea using two methods: ^{14}C-isotopic tracer incubations and dilution experiments. Because all methods of measuring growth rates may not be appropriate in all systems due to errors inherent to each method, I assessed and compared the two methods for possible sources of error by examining the effect of extended incubations on measured growth rates in ^{14}C-incubations, quantifying phytoplankton growth and grazing mortality rates through dilution experiments, and analyzing the effect of irradiance in incubations on carbon:chlorophyll ratios in dilution experiments. I found that dilution experiments yielded variable growth rates based on chlorophyll and cell abundance; the mean growth rate based on chlorophyll was 0.11 d^{-1} while mean growth rate based on abundance was 0.12 d^{-1}. Chlorophyll-based growth rates may be inaccurate due to carbon:chlorophyll ratios of phytoplankton changing during incubations. This unbalanced growth is likely due to variable mixed layer depth and subsequent variability in light history of phytoplankton. Grazing mortality rates were non-significant in 7 of the 11 dilution experiments conducted and significant mortality rates were low with a mean mortality rate of 0.09 d^{-1}, most likely because of low temperatures rather than the presence of *P. antarctica*. Growth rates measured in ^{14}C-incubations did not change in extended incubations, indicating that loss of fixed ^{14}C through grazing and respiration was not a major source of error. Growth rates were below those predicted based on temperature alone ($p<0.001$), and mean growth rate in ^{14}C-incubations was 0.14 d^{-1}. Structural equation modeling indicated that growth rates in ^{14}C-incubations did not strongly vary with mixed layer depth, but were significantly affected by low iron concentrations, most likely due to the seasonal depletion of iron. As grazing is low and physical conditions vary spatially, dilution experiments may not be an appropriate measure of growth rate in the Ross Sea, but ^{14}C-incubations yield relatively low growth rates that are significantly affected by low iron concentrations in the region.

PHYTOPLANKTON GROWTH RATES IN THE ROSS SEA, ANTARCTICA

INTRODUCTION

Phytoplankton assemblages in the Ross Sea, Antarctica

The Ross Sea is among the most productive regions in the Southern Ocean, characterized by a short growing season during which phytoplankton distribution is highly variable (Fig. 1; Arrigo et al., 2008b; Smith and Comiso, 2008). In addition to the importance of phytoplankton as the base of a diverse food web in the region, high primary production in the Ross Sea could serve as a potentially important carbon sink for the entire Southern Ocean (Arrigo et al., 2008a). The phytoplankton assemblage is dominated by diatoms and haptophytes, but dinoflagellates and cryptophytes have also been found (El-Sayed et al., 1983; Smith and Nelson, 1985; Arrigo et al., 1999). Common diatoms include pennate species such as *Fragilariopsis* spp. and *Pseudo-nitzschia* spp., which form large blooms in the region, and centric species including *Thalassiosira* spp., *Rhizosolenia* spp., and *Corethron criophilium* (Fonda Umani et al., 2002; Garrison et al., 2003).

Much of the Southern Ocean is considered to be a diatom-dominated system, but the haptophyte *Phaeocystis antarctica* serves as an additional important bloom-forming species in the Ross Sea and other regions (El-Sayed et al., 1983; Davidson and Marchant, 1986; Smith and Gordon, 1997). The presence of this species has important implications for food webs because *P. antarctica* forms large colonies that may deter grazing by some zooplankton, which in turn could restrict trophic transfer of carbon (Caron et al., 2000;

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Smith et al., 2003, 2007; Tang et al., 2008). Indeed, locations in the Ross Sea where *P. antarctica* regularly occurs have been described as a "biological waste-land" in terms of higher trophic level abundance, relative to the rest of the Ross Sea (Smith et al., 2012).

Factors limiting phytoplankton growth

Phytoplankton growth is limited by three main factors: temperature, irradiance, and nutrient supply. Eppley (1972) found temperature to be a primary factor in setting the maximum phytoplankton growth rate. He synthesized results from light-saturated, nutrient-replete cultures and established a curve indicating the upper limit of growth at specific temperatures, with maximum growth rates (μ_{max}; d^{-1}, determined by biomass changes) increasing with temperature (T; °C):

$$\log_{10}(\mu_{max}) = 0.0275T - 0.229$$

(Eq. 1)

However, only one culture with a growth temperature below 4°C was included, and none were below 2°C. Studies on the relationship between growth rate and temperature suggest that this equation may be inappropriate for temperatures typical of polar systems, as growth rates may be further suppressed at low temperatures (e.g. Goldman and Carpenter, 1974; Sakshaug and Holm-Hansen, 1986). However, Smith et al. (1999) found growth rates in the Ross Sea approached those predicted by the Eppley (1972) curve, which gives temperature-based maximum growth rates ca. 0.53-0.67 d^{-1} at temperatures typical of the Ross Sea, indicating that this relationship between temperature and growth rate is likely valid in polar regions.

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In addition to maximum phytoplankton growth rates being regulated by temperature, phytoplankton growth may also be light-limited, as phytoplankton require a minimum irradiance for photosynthesis. The Ross Sea is covered by sea ice for much of the year, and phytoplankton are likely light-limited in early spring (Smith and Gordon, 1997; Smith et al., 2000, 2013). Over the course of the spring, sea ice breaks up and melts, stratification increases due to melt-water input, and micronutrient limitation is thought to play a larger role in controlling phytoplankton growth. Although nitrogen is a limiting nutrient throughout much of the world's oceans (Moore et al., 2002), the micronutrient iron is thought to be the limiting nutrient in the Southern Ocean, an HNLC (high nutrient, low chlorophyll) region (Martin et al., 1990). Sedwick et al. (2000, 2011) found that the seasonal changes in iron concentrations likely control phytoplankton growth in the late spring and into the summer. This study measured growth rates during austral summer; therefore, it is likely that temperature is not the only factor limiting phytoplankton growth and the other limiting factors on growth (irradiance, iron) differed spatially, yielding variable phytoplankton growth rates throughout the Ross Sea.

Errors associated with measuring phytoplankton growth rates and previous growth rate measurements in the Ross Sea

Due to the spatial and temporal variability of phytoplankton blooms and the potential impact on regional carbon cycles (Arrigo et al., 2008a), a number of studies conducted in the Ross Sea have focused on factors controlling primary production, such as irradiance and micronutrient levels. Despite this focus, few studies have determined the actual growth rates of phytoplankton (Smith et al., 1999). While both growth rate and

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primary production measurements estimate the rate of biomass increase independent of losses through grazing or sinking, growth rate (d^{-1}) is the biomass-normalized instantaneous rate of biomass increase, whereas primary production (g C $area^{-2}$ $time^{-1}$) depends on the size of the standing stock of phytoplankton (Smith et al., 1999). Growth rate is a fundamental property of microbial growth and governs productivity. Growth rates of individual populations of phytoplankton ultimately control the phytoplankton assemblage in a region, which in turn affects critical oceanic processes, such as transfer of carbon through the food web, export of organic carbon from the euphotic zone, and to biogeochemical cycles (Banse, 1991; Smith et al., 1999). In addition, estimates of growth rates are critical inputs to coupled biogeochemical models (e.g., Sarmiento et al., 1998). Despite the importance of growth rates, measurements of phytoplankton growth rates in the ocean are rarely completed, largely due to methodological issues.

Although growth rates have been estimated from biomass change (e.g., Eppley, 1972), it has also been suggested that isotopic incorporation coupled with biomass estimates can be used to estimate growth rates (Eppley, 1968). Other methods have also been suggested, such as assessing the time-dependent changes of cellular pools such as ATP (Sheldon and Sutcliffe, 1978), measuring the incorporation of isotopes into protein (DiTullio and Laws, 1983), analyzing cell cycles using DNA and RNA (Carpenter and Chang, 1988), or using dilution techniques (Landry and Hassett, 1982). These methods, however, required the use of bottle incubations and are therefore susceptible to error through the "bottle effect," in which rates measured are a reflection of the artificial environment created by bottle incubations rather than the in situ environment. The bottle effect can result from changing phytoplankton and grazer assemblages over the course of

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an incubation and depletion of nutrients in the bottle (Eppley, 1968). Growth of microzooplankton and phytoplankton populations vary by group in incubations as species with a protective covering tend to have lower growth rates in bottle incubations than athecate species (Pratt and Berkson, 1959; Venrick et al., 1977; Agis et al., 2007). Dolan et al. (2000) found that in the Rhode River estuary, large tintinnid populations significantly increased while predatory ciliates and rotifer populations decreased in 24-h incubations. Venrick et al. (1977) found a shift in phytoplankton in 24-h incubations in the North Pacific central gyre; diatoms were not significantly affected by incubation, but dinoflagellates (athecate and thecate) tended to decrease, and Taguchi et al. (1993) found that pigment analysis from bottle incubations of samples from Kaneohe Bay in the Hawaiian Islands indicated a substantial shift in species composition of the phytoplankton assemblage over a 24-h incubation. Eppley (1968) demonstrated that nutrient depletion in samples collected off La Jolla, CA led to an underestimate of growth rates, especially in populations with high initial standing stocks. Macronutrient drawdown is a possible source of error in incubations conducted in regions characterized by relatively low nutrient concentrations and high biomass, but macronutrient concentrations in the Ross Sea are typically high (Martin, 1990; Landry, 1993; Arrigo et al., 2008b). Shifts in planktonic assemblages and nutrient depletion in bottles are possible sources of error in all bottle incubations, indicating that these effects must be carefully monitored in growth rate experiments.

The different manifestations of the bottle effect, as well as other possible sources of error in growth rate measurements, are likely to become more pronounced as incubation length is increased. For both isotopic tracer incubations and dilution

experiments, samples must be incubated long enough that measured growth rates will be

an accurate daily growth rate and not be significantly influenced by diel cycles (Nielsen

and Hansen, 1958), but must also be short enough to minimize bottle effects. For this

reason, typical incubation lengths for measuring growth rates are ca. 24 h (e.g. Landry

and Hassett, 1982; Smith et al., 1998; Smith et al., 1999). While previous ^{14}C-studies in

the Ross Sea have been conducted as 24-h incubations, Caron et al. (2000) determined

that 24-h incubations did not yield significant results for phytoplankton mortality (and the

associated estimates of phytoplankton growth) through dilution experiments, and

incubations needed to be as long as 72 h. Low temperatures and high macronutrient

concentrations suggest that extended incubations are not likely to alter measured

phytoplankton growth rates through bottle effects, but the effect of increasing incubation

length on measured phytoplankton growth rates has not been examined.

Errors associated with measuring phytoplankton growth rates: ^{14}C-incubations

Nutrient depletion and shifts in planktonic assemblages are possible sources of

error in growth rate measurements. However, there are additional sources of error

associated with different methods. These sources of error are unique and typically violate

the method's underlying assumptions. As such, certain methodologies for measuring

growth rates may be more appropriate in certain regions. The primary concern with

measuring growth rate using ^{14}C-incubations is the possible effect of respiration and

grazing by predators in the bottle on fixed ^{14}C, which could be a source of error where

grazing is high and phytoplankton concentrations are low (Eppley, 1980). If this were to

occur, ^{14}C-uptake would be underestimated as phytoplankton release fixed ^{14}C through

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respiration and grazers remove phytoplankton and release fixed ^{14}C through respiration

and excretion (Gieskes et al., 1979; Eppley, 1980; Moigis, 2000). This error led to

significant underestimates of growth rates in oligotrophic regions such as the North

Pacific subtropical gyre (Laws et al., 1987). Although early studies on growth rates in the

region reported rates as low as 0.08-0.14 d^{-1} (Sharp et al., 1980), the plankton rate

processes in the oligotrophic oceans (PRPOOS) program, which used ^{14}C-labeled protein

to measure relative growth rate, found that the average growth rate in the region was 1.2

d^{-1} (Laws et al., 1987), consistent with other estimates using non-tracer techniques (e.g.,

Sheldon and Sutcliffe, 1969). Whether this difference reflects an actual change in growth

rate or errors in using ^{14}C-uptake to measure growth rate was not determined, as the

sampling was not done concurrently, but Laws et al. (1987) identified zooplankton

grazing as a potential cause of the discrepancy. Subsequent studies indicate that loss of

fixed ^{14}C through grazing and respiration increases as incubation length increases, which

would lead to a decrease in measured growth rate as incubation length increases (Jackson

et al., 1983; Laws et al., 2000; Moigis, 2000). Grazing rates in the Ross Sea are

considered to be low (Tagliabue and Arrigo, 2003), in part due to low temperatures

(Caron, 2000; Rose and Caron, 2007), and respiration rates are expected to be low due to

low temperatures (Li and Dickie, 1987; Robinson and Williams, 1993; del Giorgio and

Duarte, 2002) indicating that it is less likely that this source of error would cause an

underestimate of growth rates using ^{14}C-incubations. However, the impact of bottle

effects on isotope incorporation remains uncertain.

Previous studies in the Ross Sea have used isotopic incorporation coupled with

biomass estimates to determine phytoplankton growth rates in the region. To determine

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the transformation of carbon and nitrogen through the *Phaeocystis*-dominated

assemblages in the Ross Sea, Smith et al. (1998) measured phytoplankton growth rates

using large-volume experiments (which, along with low temperatures, minimized bottle

effects). Growth rates were measured by examining changes in particulate matter

concentrations and ^{14}C-uptake, and they found that growth rates varied by method,

sampling site and time. The rates estimated ranged from 0.09 to 0.49 d^{-1}, and for the most

part remained well below the maximum growth rate defined by temperature (Smith et al.,

1998; Table 1). In an independent study, Smith et al. (1999) focused on growth rates

using uptake of independent tracers (^{14}C, ^{15}N, and ^{32}Si) to determine growth rates of Ross

Sea phytoplankton. This method yielded growth rates in summer that were lower than

those predicted based on temperature alone, and were variable based on the isotope used

(and hence the phytoplankton functional group; Table 1). Smith et al. (1999) found

carbon-based growth rates were not coupled to nitrogen-based growth rates, particularly

in the presence of *P. antarctica*. The study also looked at rate of change of biomass and

nutrients at a specific location over the course of a week to indicate phytoplankton

growth. Although growth rates measured using this method did not directly match those

found through isotopic tracer methods, the results showed similar patterns for growth

(Smith et al., 1999; Table 1). Smith et al. (2000) used ^{14}C-uptake to determine the

seasonal temporal patterns of phytoplankton growth rates in the Ross Sea and found a

strong unimodal peak in growth rates that was correlated to biomass, with mean growth

rates in spring averaging 0.27 d^{-1}. As sea ice broke up and stratification of the water

column increased, growth rates increased (Smith et al., 2000). Following the maximum in

late spring, growth rates decreased, averaging 0.059 d^{-1} in the summer, which may have

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been indicative of Fe limitation (Smith et al., 2000). While Smith et al. (1999) found

growth rates varied by method and study site, Smith et al. (2000) documented variability

in seasonal growth rates, with consistently low growth rates throughout the summer.

Errors associated with measuring phytoplankton growth rates: Dilution experiments

This study builds on these previous studies of specific growth rates in the Ross

Sea, using dilution experiments as well as isotopic incorporation to determine

phytoplankton growth rates throughout the Ross Sea in summer. The dilution technique

(e.g., Landry and Hassett, 1982; Caron et al., 2000) can be used to measure

phytoplankton mortality due to microzooplankton grazing, and estimates phytoplankton

net and intrinsic growth rates as well. Grazing is typically a major loss term for

phytoplankton, and phytoplankton and microzooplankton growth are often tightly

coupled. Previous studies have suggested that microzooplankton grazing is the largest

loss term for phytoplankton biomass in the ocean (Landry et al., 1997; Strom et al., 2001;

Calbet and Landry, 2004). For dilution experiments to accurately measure phytoplankton

mortality and growth rates, the system must adhere to the three key assumptions made in

dilution experiments: 1) phytoplankton growth is not influenced by dilution, 2) the rate of

consumption of phytoplankton by microzooplankton is directly related to the rate of

encounter, and 3) phytoplankton growth can be expressed using the exponential growth

equation (Landry and Hassett, 1982).

For the dilution method to provide an accurate measure of microzooplankton

grazing and phytoplankton growth rates, the assumptions made about growth and grazing

must be valid; however, the presence of *P. antarctica* may violate those assumptions.

Although *P. antarctica* occurs as individual cells or in mucilaginous colonies, Shields and Smith (2009) found that *P. antarctica* growth may be density dependent, with colonies growing at faster rates than individual cells under nutrient-replete conditions. Filtering the samples through 200 μm mesh to remove large grazers may disrupt colonies, altering measured growth rates. Caron et al. (2000) found that colonies broke apart during sampling and reformed over the course of the incubation, but no estimates of the effects of this treatment on growth rates was provided. Additionally, formation of colonies by *Phaeocystis* may be a mechanism to deter grazing, and grazers may avoid feeding on colonies, violating the assumption that grazing is dependent on encounter rate (Caron et al., 2000; Jakobsen and Tang, 2002; Tang et al., 2008). If the presence of *P. antarctica* colonies significantly altered grazing, a high abundance of *P. antarctica* would violate a number of the assumptions of dilution experiments, and therefore give erroneous estimates of phytoplankton growth and mortality rates.

An additional possible source of error in dilution experiments in the Ross Sea is the metric typically used to measure phytoplankton biomass in incubations: chlorophyll concentrations (Schmoker et al., 2013). Chlorophyll during incubations at a relatively constant irradiance can change due to pigment acclimation, which in turn will affect growth rate estimates (McManus et al., 1995; Landry et al., 2002; Schmoker et al., 2013). For changes in chlorophyll to provide an accurate measure of phytoplankton growth rates, growth must be balanced and the chlorophyll cell^{-1} of the phytoplankton population must remain constant, with phytoplankton producing new chlorophyll and carbon at the same rate (Gallegos and Vant, 1996). This may not hold true in lengthy incubations in regions that are heterogeneous in terms of mixed layer depth and biomass, such as the

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Ross Sea, where changes in the amount of chlorophyll cell^{-1} have been observed in dilution experiments (Caron et al., 2000). If samples are collected from the same depth and incubated at the same irradiance, but sampled from a far more variable habitat (including variable mixing or high biomass in the water column), phytoplankton may undergo different degrees of photoacclimation and alter their carbon:chlorophyll ratios (Goericke and Welschmeyer, 1992; McManus et al., 1995; Gallegos and Vant, 1996). Photoacclimation, in which phytoplankton shift carbon:chlorophyll ratios in response to changes in irradiance to optimize photosynthesis, has been demonstrated in incubations for several phytoplankton groups and results in unbalanced growth (e.g., Prézelin and Matlick, 1980; Lewis et al., 1984; Goericke and Welschmeyer, 1992). Prézelin and Matlick (1980) found that in adapting to high irradiance, phytoplankton dilute chlorophyll pools by increasing cell division rates, effectively increasing the carbon:chlorophyll ratio of the assemblage, while in response to low irradiance phytoplankton increase chlorophyll production. This response can be rapid (within 12 h; Prézelin and Matlick, 1980). In a region such as the Ross Sea, where phytoplankton biomass and mixed layer depth may vary tremendously both spatially and temporally, resulting in subsequent variability in irradiance, shifting carbon:chlorophyll ratios and unbalanced growth might be expected to occur.

Objectives of thesis

I measured phytoplankton growth rates in the Ross Sea in summer using ^{14}C-uptake and dilution experiments. My first objective was to assess and compare the different methods for measuring growth rates. To do this, I examined the effect of

incubation length on measured growth rate in ^{14}C-incubations (Eppley, 1968) and in dilution experiments (Landry and Hassett, 1982). I hypothesized that incubations can be extended to 72 h without significant shifts in phytoplankton growth rates. My second objective was to quantify phytoplankton growth rates and microzooplankton grazing rates using dilution experiments to test the hypotheses that growth rates in dilution experiments would not significantly differ from those determined using ^{14}C-incubations, and that grazing rates would be low, as in previous studies (Caron et al., 2000; Tagliabue and Arrigo, 2003; Rose and Caron, 2007). Dilution experiments were also analyzed to determine whether the presence of *P. antarctica* led to violations of the assumptions of the method and to examine possible shifts in carbon:chlorophyll ratios. My final objective was to identify the factors limiting phytoplankton growth rates, testing the hypothesis that growth rates are not limited by temperature alone and are less than the temperature-defined maximum growth rate (Eppley, 1972) in summer since phytoplankton growth is likely limited by other factors such as micronutrients (Sedwick et al., 2000, 2011; Smith et al., 2013).

MATERIALS AND METHODS

Study site and sampling procedure

Sampling for growth rate determinations was conducted in the Ross Sea during the NBP12-01 cruise from 8 January to 2 February 2012 from the *RVIB Nathaniel B. Palmer* as a part of PRISM-RS (Processes Regulating Iron Supply at the Mesoscale-Ross Sea). ^{14}C-incubations were carried out at 37 stations. Water samples were collected from 10 m using a Sea Bird 911+ CTD system containing 24 10-L Niskin bottles. Stations were sampled at the centers of two eddies (St. 4 and 14), on the Ross Bank (St. 27, 52, 75, and 76), at a low biomass region adjacent to a front (St. 9 and 22), and in the frontal regime (St. 19). Transects were also sampled near the Ross Ice Shelf and in Joides Trough (St. 56-62 and 79-92). Following the transect in Joides Trough, sampling included a high and low biomass station (Stations 94 and 93), an ice-edge station (St. 95), three stations at the center of an eddy (St. 96, 97 and 98), a station near Franklin Island (St. 101), and a station at 169°E (St. 102; Fig. 1). Water samples were collected for particulate organic carbon (POC) analyses at all stations sampled for ^{14}C-incubations.

Dilution experiments were conducted at 11 of the 37 stations where ^{14}C-incubations were conducted (Fig. 1). Water from 10 m was used for incubations, and was gently filtered through 200 µm mesh screen to remove any mesozooplankton. Further processing took place in a cold room (0°C).

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Analytical methods

Temperature at 10 m depth was determined from vertical temperature profiles obtained from conductivity-temperature-depth (CTD) measurements. Mixed layer depth estimates were based on the water density profiles of the CTD up-casts and were determined based on change in potential density (σ_t) of 0.01 unit from the σ_t value at 10 m (Thomson and Fine, 2003).

Chlorophyll *a* concentrations were determined fluorometrically. Chlorophyll samples were filtered under low vacuum through 25 mm Whatman GF/F filters, placed in 90% acetone, and extracted for at least 24 h in cold and dark conditions; chlorophyll was measured on a Turner Designs TD-700 fluorometer (JGOFS, 1996). The fluorometer was calibrated using commercially prepared chlorophyll *a* (Sigma). For particulate organic carbon (POC) measurements, 0.25 – 1.0 L of water was filtered under low vacuum through combusted (450°C for 2 h) Whatman GF/F filters. All filters were rinsed with ca. 5 mL of 0.01N HCL in filtered seawater (to remove inorganic carbon), placed in combusted glass vials capped with combusted aluminum foil, and dried at 60°C. The POC samples were analyzed in the laboratory via pyrolysis on a Costech ECS 4010 elemental analyzer. Blanks were filters through which filtered seawater had been run (ca. 5 mL) and treated in the same manner (Gardner et al., 2000).

Whole seawater samples were preserved in acid Lugol's and analyzed in the laboratory for phytoplankton composition. Subsamples ranging from 10-50 mL (volume based on chlorophyll concentrations) were settled in a Utermöhl counting chamber for a minimum of 24 h (Utermöhl, 1931). If phytoplankton and microzooplankton

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concentrations were low, samples were resettled using a larger chamber. Following the 24-h settling period, samples were examined at 250 and 400x with an Olympus CKX41 inverted microscope. Samples were settled and analyzed at least twice using both magnifications. Microzooplankton were categorized into four groups: aloricate ciliates, loricate ciliates, and small (< 20 μm) and medium (> 20 μm) dinoflagellates. Microzooplankton were not classified to genus level. Acid Lugol's preservation does not allow for designation of dinoflagellates into autotrophic, mixotrophic, or heterotrophic categories. As many species of dinoflagellates have been found to be mixotrophic (Flynn et al., 2013), dinoflagellates were initially classified as microzooplankton, but were treated as phytoplankton for later calculations of growth rates.

Phytoplankton were identified to genus, or where possible, species level. Species present included several species of diatoms, the prymnesiophyte *Phaeocystis antarctica* in colonial (unflagellated, encased in an envelope) and solitary (flagellated) form, and the silicoflagellate *Dictyocha speculum*. The entire contents of the Utermöhl chamber were counted except solitary *P. antarctica* and the diatom *Cylindrotheca closterium*, which were the most numerically abundant species in all samples (often >1000 cells mL^{-1}). *P. antarctica* in colonial form was enumerated using the Utermöhl chamber, but abundance was analyzed by counting all cells present in colonies.

Due to the high abundance of solitary *P. antarctica* and *C. closterium*, these species were enumerated using a Sedgwick-Rafter counting chamber. Samples were well-mixed and transferred to the counting chamber using a Pasteur pipette where they were then allowed to settle for a minimum of 15 minutes before examination under an

Olympus CKX41 inverted microscope. Three transects of thirty grids were analyzed for species abundance (Ehrlich, 2010).

Counting precision was estimated for all species and samples to a 95% confidence limit by:

$$\text{Counting error (\%)} = 100 \times \frac{2}{\sqrt{n}}$$

where n is the number of cells counted of each species (Lund, 1958; Hotzel and Croome, 1999). For rare species, such as the diatoms *Corethron criophilum* and *Thalassiosira* spp., counting error was often greater than 100%, and these rare species were not used for determination of growth rates based on abundance.

The sizes of species present in relatively high concentrations were estimated to determine average cell volume and carbon content. For each of the species, at least 50 cells were measured to determine average length and width. The running standard deviation and coefficient of variation were determined for each dimension to ensure that the number of cells provided an accurate representation of the variation within the species. Average cell volume of each species was then determined from the average length and width of that species and applying formulas for their closest geometric shape. Volumes were then converted to carbon based on classification using the following volume (μm^3) to carbon (pg C) models:

- diatoms: log pg C = 0.76 log volume - 0.352 (Eppley et al., 1970; Smayda, 1978);

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- prymnesiophytes: log pg C = 0.899 log volume – 0.642 (Menden-Deuer and Lessard, 2000);

- dinoflagellates: log pg C = 0.864 log volume – 0.353 (Menden-Deuer and Lessard, 2000);

- others: pg C = volume x 0.08 (Beers and Stewart, 1970).

Carbon values were then converted to carbon biomass by species using cell count data. Calculated cellular biomass was comparable to phytoplankton biomass estimates previously seen in the Weddell and Ross Seas (Mathot et al., 2000; Kang et al., 2001).

[14]C-incubations

Sample water was distributed into triplicate 345 mL Qorpak tissue culture flasks and each was inoculated with 20-40 µCi ^{14}C-bicarbonate, capped, and gently inverted several times. Qorpaks were then placed in a simulated in situ incubator cooled with running seawater and screened to 36% of surface irradiance to simulate irradiance at 10 m. After 24 h the first Qorpak was removed, and a 100 µl subsample placed in a 10 mL scintillation vial with 100 µl β-phenethylamine (a CO_2 trap) and 5 mL Ecolume[®] fluor for the determination of the total isotope addition. The remaining sample was divided into 100 and 245 mL portions and filtered through 25 mm GF/F filters. Filters were rinsed with ca. 5 mL 0.01N HCl in filtered seawater and placed in scintillation vials with 5 mL Ecolume[®]. After 24 h samples were analyzed for isotope incorporation using a liquid scintillation counter (Beckman). The remaining two Qorpaks were processed as described above, one after 48 h and the last after 72 h.

Primary production was determined for each sample and time point, and these

data were then converted to growth rate. Primary production was calculated using the

equation

$$PP = \frac{(24000)*(1.05)*(\Delta DPM)*\left(\dfrac{V_T}{V_F}\right)}{(DPM_{Total}*10)*V_T*t}$$
(Eq. 3)

where ΔDPM is the uptake of isotope (disintegrations per minute) during the incubation,

V_T is the total volume incubated (345 mL), V_F is the volume filtered (100 or 245 mL),

DPM_{Total} is the average activity of the triplicate measurements of isotope additions

(disintegrations per minute), and t is incubation length (h). In this equation, 24,000 is the

weight of inorganic carbon (mg m^{-3}) and 1.05 is an isotope discrimination factor (Eppley,

1968). Daily growth rates (d^{-1}) were calculated using POC concentrations and the method

of Eppley (1968):

$$\mu = \frac{1}{t}\ln\left(\frac{P_0 + \Delta P}{P_0}\right)$$
(Eq. 4)

where P_0 is the initial concentration of particulate organic carbon (μg L^{-1}), ΔP is the

change in organic carbon (μg L^{-1}) over the course of the incubation, and t is the

incubation length (d).

To determine the factors influencing measured growth rates, data were first

transformed using a natural log transformation to fit the assumptions of normality and

homogeneity of variance. This transformation was completed using the Box-Cox

transformation procedure (Neter, 1996). Growth rates from 24-h incubations were

compared to the temperature-defined maximum growth rates (Eppley, 1972) using a one-

tailed t-test ($\alpha=0.05$) to determine significant deviations from this theorized rate.

Measured growth rates at 24 h were tested for regression with initial biomass, both in

terms of POC and initial chlorophyll concentrations, which were also log-transformed

based on Box-Cox transformation procedure, to evaluate the relationship between

standing stock and growth rate (least-squares regression, $\alpha=0.05$). The effect of

incubation length was analyzed using analysis of covariance (ANCOVA; $\alpha=0.05$)

blocking by initial biomass based on POC (μg C L^{-1}).

The relationships among growth rates and environmental parameters (e.g.,

irradiance and iron concentrations) were analyzed using structural equation modeling

(SEM; e.g. Anderson and Gerbing, 1988; Grace et al., 2010). The model tested included

interactions between temperature, mixed layer depth, iron concentrations, POC, and

initial carbon:chlorophyll ratios. The model was analyzed to determine the relative roles

of irradiance, iron availability, and temperature on measured growth rates. Relationships

were based on known possible interactions between parameters and the number of

parameters was analyzed to ensure that the model was not under- or over-identified. As

the model was used for theory testing and run with observed data, maximum likelihood

estimation was used (Anderson and Gerbing, 1988). The fit of the model was evaluated

using several fit criteria ($\chi^2 > 0.05$ and Root Mean Square Error of Approximation <

0.05), and the relationship between each environmental parameter and growth rate was

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analyzed using the standardized path coefficients. This analysis was run using the *lavaan* package in R.

Dilution experiments

Eleven stations were sampled for dilution experiments, three of which were sampled as time-course experiments. All experiments were conducted using a modification of the two-point dilution technique. This method typically uses a 100% whole seawater (wsw) treatment, which contains all organisms less than 200 μm in size and is representative of net growth rate, and a 5% wsw treatment that theoretically decreases the rate of encounter between phytoplankton and grazers to zero, reducing grazing mortality to zero. This 5% wsw treatment thus represents intrinsic growth rate (Strom et al., 2006; Landry et al., 2008; Strom and Fredrickson, 2008). Previous studies comparing two-point dilutions with a dilution series (5 dilution treatments) showed that both gave similar growth rate estimates (Strom et al., 2006; Strom and Fredrickson, 2008; Li et al., 2011). It is not always feasible to use a 5% wsw treatment as changes in chlorophyll at this dilution level may be undetectable. As such, a 20% wsw treatment was used to keep chlorophyll concentrations at detectable levels and for the measurement of significant grazing (Menden-Deuer and Fredrickson, 2010).

To conduct dilution experiments, water was first filtered through a 0.2 μm Whatman cartridge filter to generate filtered seawater, and untreated (but filtered through a 200 μm net) seawater was gently added to achieve a final concentration of 20% wsw. The total volume filtered depended on whether the experiment was to be conducted as a time course. Three 1.2-L bottles were collected from the 20% and 100% wsw treatments

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for each time point for a total of 6 1.2-L bottles for non-time course experiments and 18 1.2-L bottles for time courses. The 1.2-L bottles were placed in the incubator screened to simulate light intensities of 36% of surface irradiance. Nutrients were not added to the carboys, as the Ross Sea is typically characterized by high concentrations of macronutrients (Sedwick et al., 2000; Smith et al., 2000). Micronutrients (Fe) were not added as sampling and experiments were not trace-metal free. For non-time course experiments, all bottles were processed after 72 h (Caron et al., 2000). Three experiments were conducted as time-course experiments, and samples were collected from each treatment after 24, 48, and 72 h to assess how phytoplankton mortality and growth rates in dilution experiments varied across incubation length. At each time point, a 250 mL sample was preserved in acid Lugol's (final concentration of 10%) for microscopic analysis from each 100% wsw treatment and triplicate 250 mL samples were analyzed for chlorophyll determinations.

Chlorophyll was used was a proxy for biomass to determine growth rate (k) using the exponential growth equation (Landry and Hassett, 1982):

$$k = \left(\frac{1}{t}\right) \ln\left(\frac{N_t}{N_0}\right) \qquad \text{(Eq. 5)}$$

where t is the length of incubation (d), N_t is chlorophyll concentration after incubation, and N_0 is chlorophyll concentration prior to incubation. Growth rates were obtained for all samples, and a two-tailed t-test was used to determine if growth rates in the 100% wsw treatment were significantly different (α=0.05) from growth rates in the 20% wsw

treatment. If growth rates did not differ significantly among treatments, grazing mortality was described as not significantly different between treatments (NS). If growth rates were significantly lower in the 20% wsw sample, signifying a negative grazing rate (which is theoretically impossible, but has been observed previously in dilution experiments), all statistical tests were conducted with non-significant and negative grazing rates included but set to zero (Calbet and Landry, 2004; Menden-Deuer and Fredrickson, 2010). For stations with a significant difference in net growth rates between treatments, grazing mortality (m) was calculated by

$$m = \frac{k_d - k}{1 - D}$$ (Eq. 6)

where k_d is growth rate in the 20% wsw treatment, k is growth rate in 100% wsw treatment, and D is the fraction undiluted seawater (Landry and Hassett, 1982; Li et al., 2011). Intrinsic growth rate (μ) was calculated for those stations with significantly positive grazing rates using the equation

$$\mu = \frac{k_d - kD}{1 - D}$$ (Eq. 7)

(Landry and Hassett, 1982; Li et al., 2011). Intrinsic growth rates at stations that did not have a significantly positive grazing rate were categorized as not available (NA).

To determine the factors influencing measured growth and grazing rates from dilution experiments, net growth rates based on fluorometric analysis were tested for

normality (Shapiro-Wilks; α=0.05), and were analyzed without transformation. Since only three experiments yielded significant grazing rates that could be used to determine intrinsic growth rates, all statistical tests were run using net growth rates. Growth rates from 72-h incubations were compared to temperature-defined maximum growth rate using a one-tailed t-test (α=0.05) to determine if measured growth rates were below maxima based on temperature (Eppley, 1972). Measured growth rates were tested for correlations with environmental variables (temperature, nutrient concentrations, and mixed layer depth) as well as initial biomass (chlorophyll and POC concentrations; Pearson correlation; α=0.05).

Carbon biomass based on microscopic analysis was summed for each sample and net growth rates based on total carbon concentration calculated for all stations using the exponential growth rate equation. Net growth rates based on cell abundance were tested for normality (Shapiro-Wilks; α=0.05), and analyzed without transformation. Measured growth rates were tested for correlations with temperature, nutrient concentrations, and mixed layer depth as well as initial biomass (chlorophyll and POC concentrations; Pearson correlation; α=0.05). Relative biomass of each phytoplankton functional group (diatoms, prymnesiophytes, dinoflagellates, and silicoflagellates) and relative abundance of each microzooplankton functional group (dinoflagellates, silicoflagellates, aloricate ciliates, and loricate ciliates) were compared between initial and final time points to determine if there was a shift in assemblage composition over the course of the incubation (two-tailed t-test; α=0.05)

To determine whether there were shifts in the carbon:chlorophyll ratios during incubations, carbon:chlorophyll ratios were calculated using total carbon concentrations

derived from microscopy and average chlorophyll concentrations at the corresponding time point. Per cent changes in carbon:chlorophyll ratios were then calculated based on the difference in calculated ratios in initial and final samples to determine if ratios changed during incubation. Measured changes were tested for correlation with temperature and mixed layer depth as well as initial biomass (Pearson and Spearman correlations; $\alpha=0.05$).

Comparison of growth rates across methods

To compare growth rates measured within 72-h dilution experiments, growth rates by cell abundance were compared against growth rates estimated from chlorophyll concentrations (Pearson correlation; $\alpha=0.05$). Because samples were taken from the same bottle, least-squares regression should yield a significant positive regression ($\alpha=0.05$); increases in chlorophyll-based growth rates should correlate with increasing abundance-based growth rates under balanced growth (in which chlorophyll and carbon increase at the same rate). Growth rates were compared between ^{14}C-incubations and dilution experiments to determine if the two methods were significantly different. Because growth rates were not expected to be normally distributed, growth rates across treatments were compared using a Friedman test ($\alpha=0.05$) blocking by initial biomass (μg Chl *a* L^{-1}).

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RESULTS

Phytoplankton assemblage composition

Initial chlorophyll concentrations varied across all stations at the start of dilution

experiments, ranging from 0.22-9.45 µg L^{-1}, a result of the high variability in abundance

and biomass of phytoplankton across all stations sampled (Fig. 2). Diatoms contributed

the most to total phytoplankton biomass at all but one station, with an average biomass of

102 µg C L^{-1} (Fig. 3). Of the diatoms observed, the small pennate diatom *Cylindrotheca*

closterium was the most abundant, ranging from 14 to 7,900 cells mL^{-1}, and constituting

up to 97% of the total phytoplankton assemblage (Fig. 4). Other diatom genera occurring

in significant concentrations included the pennates *Fragilaropsis*, *Pseudo-nitzschia*, and

Nitzschia and centrics *Dactyliosolen* and *Chaetoceros* (Fig. 3). *Fragilariopsis* spp. and

Chaetoceros spp. were present in higher concentrations (relative to cells of *Cylindrotheca*

closterium) at the two Ross Ice Shelf stations (St. 60 and 61; Fig. 4). Larger diatoms

(*Corethron criophilum*, *Thalassiosira* spp., and *Rhizosolenia* spp.) were present, but

never made up a significant fraction of phytoplankton abundance or biomass, and cells

were rarely intact. *P. antarctica* was either the most abundant or second most abundant

species at the majority of stations sampled (Fig. 4), but contributed the lowest average

biomass (1.1 µg C L^{-1}) across the sampling region due to the relatively low cellular

carbon content (Fig. 3; Table 2). Solitary forms were consistently more abundant than

colonies, although colonies were present at most stations (Fig. 4). The highest

concentration of colonial *P. antarctica* was seen at the eddy center station (St. 4), but

high concentrations were also seen at the Ross Ice Shelf stations (St. 60 and 61; Fig. 4).

Of planktonic groups that have demonstrated heterotrophic behavior,

dinoflagellates were present in relatively high concentrations at the eddy center (St. 4;

Fig. 4). Small dinoflagellates (<20 μm) were on average more abundant than medium

dinoflagellates (>20 μm; Fig. 5). Dinoflagellate abundance was high at St. 3 and a Ross

Ice Shelf station (St. 61), and at both Joides Trough stations (St. 79 and 80; Fig. 4).

Concentrations of *Dictyocha speculum* were also highest at the two Ross Ice Shelf

stations (St. 60 and 61) and this species was present only at one additional station (St. 37;

Figs. 3 and 4). Aloricate ciliate abundance ranged from 2,000 to 19,000 cells L^{-1}, and

average abundance was 12,300 cells L^{-1} (Fig. 5), but was not correlated with

phytoplankton biomass (Pearson correlation; $p>0.05$). Of all functional groups, small

dinoflagellates were the only group that showed significantly higher biomass at high

phytoplankton biomass stations ($r^2=0.52$).

Phytoplankton mortality and growth rates in dilution experiments

Phytoplankton mortality rates were only significantly different between

treatments in 4 of the 11 dilution experiments, and were significantly greater than zero in

only 3 of those 4 (Table 3). A mortality rate significantly less than zero (-0.20 d^{-1})

occurred at the second occupation of the low biomass station (St. 22), and the highest

mortality rate (0.30 d^{-1}) occurred at the initial occupation of this station (St. 9) three days

earlier (Table 3). Significant mortality rates were also measured at the outside ice station

(St. 3; m=0.18 d^{-1}) and a Joides Trough station (St. 80; m=0.09 d^{-1}; Table 3). The three experiments that yielded significant and positive mortality rates occurred at stations with relatively low initial biomass (0.33-1.08 μg chl a L^{-1}), but there was no significant correlation between initial biomass and phytoplankton mortality rates (Pearson correlation; p>0.05). At the stations with significant phytoplankton mortality rates, net growth rates based on chlorophyll and cell abundance at the stations with significant phytoplankton mortality rates ranged from -0.02 to 0.26 d^{-1} and 0.10 to 0.16 d^{-1}, respectively, but there was no significant correlation between either measure of growth rates and phytoplankton mortality rates (Pearson correlation; p>0.05; Fig. 6). Intrinsic growth rates based on net growth rates from chlorophyll and cell abundance at stations exhibiting significant positive phytoplankton mortality rates ranged from 0.08 to 0.56 d^{-1} and 0.19 to 0.43 d^{-1}, respectively (Table 3).

There was no relationship between *P. antarctica* and phytoplankton mortality rates; mortality rates did not differ significantly with increasing abundance or biomass of colonial *P. antarctica* (Fig. 6). Colonial *P. antarctica* was present at stations that yielded significant mortality rates and was absent from several stations that did not yield significant mortality rates (Fig. 6). There was also no relationship between total *P. antarctica* (colonial or solitary forms) biomass and abundance and phytoplankton mortality rates.

Dilution experiments conducted as time-courses at the eddy center station (St. 4), frontal station (St. 22), and one Joides Trough station (St. 79) yielded variable results, possibly in part due to high variability in phytoplankton biomass at the time of sampling. Standing stocks at the time-course stations ranged from 0.22 μg chl a L^{-1} (St. 79) up to

9.45 µg chl a L^{-1} (St. 4), an order of magnitude difference. The dilution experiment at one

station (eddy center; St. 4) did not yield significant grazing rates at any time point while

the dilution experiment at one station (St. 22) yielded significant phytoplankton mortality

rates in both 48- and 72-h incubations, and the dilution experiment at another station (St.

79) yielded significantly negative phytoplankton mortality rates only at 24 h (Table 3).

Net growth rates based on both chlorophyll and cell abundances were negative in 24-h

incubations (Fig. 7), and net growth rate was typically higher in 48 h incubations than in

72 h incubations but the relationship between growth rates at 48 h and 72 h also differed

by station (Fig. 7). One station (St. 4) yielded negative chlorophyll-based growth rates

throughout the incubation while two stations (St. 22 and 79) yielded positive chlorophyll-

based growth rates throughout. All stations yielded negative cell abundance-based growth

rates at 24 h and positive cell abundance-based growth rates at 72 h, but abundance-based

growth rates at 48 h differed by station (Fig. 7).

Net growth rates in dilution experiments differed between abundance-based

growth rates and chlorophyll-based growth rates, but were low throughout the sampling

period. Growth rates measured both by chlorophyll and cell abundance were lower than

the temperature-defined maximum growth rate (Eppley, 1972) (t-test; $p<0.001$). Although

net growth rates based on chlorophyll did not differ significantly from net growth rates

based on cell abundance (paired Wilcoxon Signed Rank test; $p>0.05$), there was no

correlation between rates measured from the two methods (Pearson correlation; $p>0.05$),

and net growth rates based on chlorophyll ranged from -0.12 to 0.47 d^{-1} while net growth

rates based on cell abundance ranged from 0.05 to 0.20 d^{-1} (Fig. 8). A comparison of

growth rates from the two methods indicated that the relationship between the two may

be negative although the trend was not significant (p>0.05), as high net growth rates based on chlorophyll were associated with low net growth rates based on cell abundance (Fig. 8). There were also different relationships between growth rates and environmental conditions depending on the method. Net growth rates based on chlorophyll increased with increasing temperature (r^2=0.87; Fig. 9), but high net growth rates as measured by chlorophyll were also associated with samples with high initial carbon:chlorophyll ratios (r^2=0.77) and shallow mixed layer regions (Spearman correlation; P= -0.72; Fig. 9). Net growth rates based on cell abundance data were not correlated with any environmental factor (Fig. 9), and neither measure of growth rate yielded a significant relationship between net growth rate and standing stocks (Pearson correlation; p>0.05).

The carbon:chlorophyll ratios shifted in all samples examined microscopically (Fig. 10). Carbon:chlorophyll ratios in samples taken at the start of experiments ranged from 3.9 to 165, while the range in carbon:chlorophyll ratios in samples from 72-h incubations was narrower (5.9-85.6). The changes in carbon:chlorophyll ratios from initial to final time points was significantly correlated with temperature, mixed layer depth, and initial carbon:chlorophyll ratios. Samples from stations with low temperatures and deeper mixed layers (which also had lower carbon:chlorophyll ratios at the start of the incubation; Fig. 10) showed a significant positive shift in carbon:chlorophyll ratios during incubation (r^2=0.65 for temperature and Spearman correlation; P=0.71 for mixed layer depth; Fig. 10). The strong correlation between temperature and mixed layer depth (Spearman correlation; P= -0.76) and the relationship between carbon:chlorophyll ratios and mixed layer depth indicate that chlorophyll-based growth rates may be strongly influenced by changing cellular pigment concentrations over the course of the incubation

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as a result of differences in light regimes between the water column and incubators. Biomass contributed by diatoms, dinoflagellates (>20 μm and <20 μm), *P. antarctica*, and silicoflagellates did not significantly change during incubations (two-tailed t-test; p>0.05) and relative abundance of dinoflagellates (>20 μm and >20 μm), silicoflagellates, aloricate ciliates, and loricate ciliates did not significantly change during incubations (two-tailed t-test; p>0.05).

Growth rates in ^{14}C-incubations

Growth rates measured in ^{14}C-incubations were low throughout the sampling period, but were not significantly different from those measured in dilution experiments (Friedman test; p>0.05) and similar to summer growth rates measured in previous studies (Tables 1 and 4). Growth rates were variable, with rates in 24-h incubations ranging from 0.03 to 0.85 d^{-1}, with a mean of 0.14 d^{-1} (Table 4). The measured growth rate did not vary with increasing incubation length (ANCOVA; p>0.05) and decreased with increasing initial POC concentrations for all incubation lengths (r^2=0.19; Fig. 11). Growth rates were low in regions with high initial POC concentrations (>40 μM), indicating these regions may have exhausted available resources after reaching high phytoplankton biomass (Fig. 11). Although regions with lower initial biomass had higher growth rates, these growth rates were still significantly lower than the temperature-based maximum growth rates (Eppley, 1972; p<0.001).

Since measured growth rates were consistently less than those predicted based on temperature, phytoplankton growth was likely primarily limited by some other environmental factor if the Eppley (1972) relationship applies to growth rates at low

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temperatures. Based on previous studies in the Ross Sea, the most likely limiting factors are irradiance and iron concentrations. A structural equation model was built to test the relationships between measured phytoplankton growth rates, irradiance, and iron using the interactions between temperature, mixed layer depth, iron concentrations, POC, and initial carbon:chlorophyll ratios (Fig. 12). Mixed layer depth, POC, and initial carbon:chlorophyll ratios provide insights into irradiance in the water column, as deep mixed layers and high POC can both reduce the mean irradiance experienced by phytoplankton. The carbon:chlorophyll ratio is potentially indicative of the irradiance history of the phytoplankton assemblage (Fig. 12), although carbon:chlorophyll is also influenced by available iron as iron is used to synthesize chlorophyll in response to reduced irradiance. Mixed layer depth can also affect iron concentrations, with deeper mixing potentially introducing iron to the surface layer, and POC can influence iron concentrations via rapid removal of iron during growth (Fig. 12). The model fit well with observations at the stations at which ^{14}C-growth rates were measured, as the covariance matrix based on the model run did not significantly differ from the covariance matrix of the observed data (p-value (χ^2) =0.595; RMSEA<0.05).

The model results indicate that the strongest effects on measured growth rate are linked to iron and POC concentrations (Fig. 13). Iron concentrations in the austral summer in the Ross Sea typically fall below 0.2 nM in near-surface waters (Sedwick et al., 2011) and were low throughout the sampling period (Sedwick et al., in prep; Appendix 1), and structural equation modeling indicates that an increase in iron concentrations by 1.0 standard deviation would lead to an increase in growth rates by 0.5 standard deviation (e.g. a 0.12 nM increase in iron concentrations would lead to an

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increase in growth rate of 0.07 d^{-1}; Fig. 13). Additionally, an increase in POC

concentrations by 1.0 standard deviation would generate a decrease in growth rate by

0.31 standard deviations (Fig. 13). Changes in mixed layer depth have a greater impact

on growth rates than changes in temperature (Fig. 13), and although iron concentrations

increased with increasing mixed layer depth, the relationship between iron and POC

concentrations was stronger (Fig. 13). Additionally, the relationship between iron

concentrations and carbon:chlorophyll ratios was stronger than the relationship between

mixed layer depth and carbon:chlorophyll ratios (Fig. 13). The variance in growth rate is

well explained by this simple model; excluding the effects of POC (it is only included as

an intercorrelation), the model predicts 31% of the variance in growth rates (Fig. 13).

Although this model does not include all possible factors affecting irradiance and iron

concentrations, and results are limited to the 37 stations sampled for growth rate by ^{14}C-

incubations, the model indicates that iron likely played an important role in regulating

phytoplankton growth rates.

DISCUSSION

Although it is clear that phytoplankton growth plays a critical role in ocean processes, phytoplankton growth rate measurements are subject to errors resulting from incubation and experimental technique, leading to few measurements of growth rates in polar waters. Based on the designs of the methods used to estimate growth rates, some procedures may be more appropriate than others, and it is important that any flaws or uncertainties inherent to a certain method be acknowledged and, when possible, quantified when measuring growth rates. The dilution method and ^{14}C-incubations rely on key assumptions concerning grazing in the bottle incubations; for the dilution method to provide reliable estimates, phytoplankton should not deter grazing by microzooplankton; similarly, if ^{14}C-incubations accurately measure growth rates, grazing in the bottles should be quantitatively unimportant. Additionally, while ^{14}C-incubations and growth rates based on cell abundance measure changes in carbon, growth rates estimated from dilution experiments typically rely on changes in chlorophyll, which requires that the system exhibit balanced growth (i.e. carbon and chlorophyll increase at the same rate). Previous growth rate studies in the Ross Sea (e.g. Smith et al., 1999) found variable phytoplankton growth rates depending on the method used; furthermore, they were dependent on time of year of sampling and whether the population was composed of diatoms or *P. antarctica* (Table 1).

Photoacclimation in dilution experiments

Phytoplankton growth rates in this study varied based on the method used. In dilution experiments, growth rates based on changes in chlorophyll were negative at stations that were characterized by low temperatures and deep mixed layers. It is not possible for ^{14}C-incubations to yield negative growth rates, but growth rates based on cell abundance, which were taken from the same samples used to measure growth rates based on changes in chlorophyll, were always positive and relatively constant throughout the sampling period. There are errors associated with measuring growth rates from cell abundance data; among these are that picophytoplankton were not counted, cell volume was calculated from approximate geometric shapes (potentially under- or overestimating the average cell volume), cell volume may shrink after preservation (Montagnes et al., 1994), and error associated with cell carbon from volume conversions. However, the discrepancy between chlorophyll-based growth rates and abundance and ^{14}C-based growth rates in this study is likely due to unbalanced growth in dilution experiments due to photoacclimation, in which phytoplankton under increased irradiance in incubators may have increased productivity and cell division rates while the chlorophyll pools remained unchanged (resulting in increased carbon:chlorophyll ratios) and phytoplankton under decreased irradiance increased chlorophyll levels (resulting in decreased carbon:chlorophyll ratios; Prézelin and Matlick, 1980). This would have resulted in a disproportionate increase or decrease in chlorophyll concentrations relative to total phytoplankton carbon, similar to the unbalanced growth seen in the dilution experiments conducted by Caron et al. (2000).

Based on the relationship between the initial carbon:chlorophyll ratio and shifts in carbon:chlorophyll ratio during dilution experiments, it appears that altered chlorophyll cell^{-1} concentrations due to variable irradiance may have had a substantial effect on measured growth rates. Negative chlorophyll-based growth rates occurred at stations characterized by low initial carbon:chlorophyll ratios, suggesting that samples taken from these stations were adapted to relatively low photon flux densities. At stations with deep mixed layers, chlorophyll-based growth rates were negative, although abundance-based growth rates remained positive, indicating that a decrease in chlorophyll cell^{-1} occurred. In contrast, experiments at stations with shallow mixed layers had high initial carbon:chlorophyll ratios, typical of a phytoplankton assemblage acclimated to high irradiances. In response to conditions in the incubator, phytoplankton taken from these stations manifested decreased carbon:chlorophyll ratios over the incubation, which would be expected in response to decreased irradiance (Prézelin and Matlick, 1980). Even though samples were taken from the same depths and were incubated under 36% of surface irradiance, the relationship between mixed layer depth and carbon:chlorophyll ratios indicates that unbalanced growth occurred and varied across the sampling region, and previous studies have indicated that irradiance experienced by phytoplankton in the water column is difficult to replicate in ship-board incubations (McManus, 1995). These experiments were conducted as extended incubations, but photoacclimation can occur rapidly (within 12 h in cultured samples; Prézelin and Matlick, 1980). In a heterogenous region such as the Ross Sea, where irradiance varies temporally, spatially and with depth, studies assessing growth rates using chlorophyll concentrations need to carefully quantify

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shifts in chlorophyll cell^{-1} in response to changing irradiance, since photoacclimation could lead to spurious growth rates.

Phytoplankton mortality rates in dilution experiments

Dilution experiments yielded phytoplankton mortality rates that were extremely low, but similar to those seen previously in the Ross Sea (Caron et al., 2000), suggesting that grazing by microzooplankton is not a large source of error in ^{14}C-incubations. The majority of the stations sampled yielded phytoplankton grazing mortality rates that did not significantly differ between treatments; furthermore, the rates that were significant were low compared to rates measured in the Sargasso Sea (e.g. Lessard and Murrell, 1998), but not unusual for polar regions (Caron et al., 2000; Garzio and Steinberg, 2013; Garzio et al., in press). The low rates could not be directly attributed to low abundance of ciliates, as ciliate abundance was within the range seen in the Sargasso Sea (Lessard and Murrell, 1996), and ciliate abundance was also similar to abundances reported previously in Southern Ocean studies (Caron et al., 2000; Garzio et al., in press). Dennett et al. (2001) found that the relative contribution of phototrophic dinoflagellates to overall abundance of dinoflagellates in the Ross Sea varied substantially from almost exclusively phototrophic dinoflagellates to exclusively heterotrophic forms. While this study grouped heterotrophic and phototrophic dinoflagellates, total concentrations of dinoflagellates fall within the range of heterotrophic dinoflagellate abundance seen in previous Southern Ocean studies (Caron et al., 2000; Garzio and Steinberg, 2013; Garzio et al., in press). Although heterotrophic dinoflagellate abundance was not quantified, previous studies

from this region were unable to link low phytoplankton mortality to microheterotroph abundance (Caron et al., 2000).

Previous studies were also unable to attribute low grazing mortality to high phytoplankton abundance and subsequent reductions in clearance rates of microzooplankton (Landry, 1993; Caron et al., 2000). This effect could lead to a significant source of error in a two-point dilution; if microzooplankton feeding reached a threshold at phytoplankton abundances below 100% wsw treatments, measured phytoplankton grazing mortality rates would be underestimated compared to net growth across the two treatments. If a threshold effect occurred in these dilution experiments, it could have lead to underestimates of phytoplankton mortality rates at stations with high initial biomass (Calbet et al., 2011). This threshold effect could explain why significant grazing mortality rates occurred at stations with low phytoplankton standing stocks; however, there was no direct relationship between initial biomass and grazing mortality, and several stations with low initial biomass did not yield significant grazing mortality rates. In fact, while the low biomass station (St. 9) had the highest grazing mortality observed, grazing mortality during the second occupation (during which initial biomass was lower than it was at the first occupation) was significantly *less than* zero. The lack of any clear relationship between initial biomass and phytoplankton grazing mortality rates and results of previous dilution experiments conducted under similar conditions (Caron et al., 2000) indicate that it is unlikely that low grazing mortality rates are due to high prey concentrations.

Alternative explanations for these extremely low measured phytoplankton mortality rates in the Ross Sea include depression of microzooplankton herbivory at low

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temperatures and error in measuring phytoplankton grazing mortality rates due to the presence of *P. antarctica*. Previous dilution experiments in the Southern Ocean have found a positive relationship between temperature and phytoplankton grazing mortality rates as low temperatures yielded low grazing mortality rates (Burkill et al., 1995; Archer et al., 1996; Schmoker et al., 2013; Garzio et al., in press). An analysis by Caron et al. (2000) on 19 previous dilution experiment studies found that those experiments conducted at low temperatures (<2°C) found significantly lower phytoplankton mortality rates than those at high temperatures (>10°C). Rose and Caron (2007) found that growth rates of herbivorous protists in high-latitude systems declined faster than those of phototrophic protists when assessed relative to temperature, further indicating that the low phytoplankton mortality rates could result from low temperatures.

Several studies conducted in regions characterized by high abundances of *Phaeocystis* have found low phytoplankton mortality and growth rates, leading to the hypothesis that the presence of *Phaeocystis* may violate assumptions of dilution experiments. Caron et al. (2000) hypothesized that *P. antarctica* may contribute to low grazing mortality, but also found that *P. antarctica* was unlikely to be the only source of low phytoplankton mortality rates in the region. In the Arctic Calbet et al. (2011) found extremely low phytoplankton growth rates, as well as low grazing mortality rates in a region dominated by solitary and colonial *Phaeocystis pouchetii*. They hypothesized that these low rates were caused by chemicals released by *P. pouchetii* that interfered with grazing activity and allelopathically interacted with other phytoplankton in the incubations, suppressing growth (Calbet et al., 2011). Additionally, they hypothesized that *P. pouchetii* evaded grazing when in colonial form, and colonies lead to variability in

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the distribution of phytoplankton in the bottles that in turn affected chlorophyll measurements in the experiments (Calbet et al., 2011). However, their sampling did not allow them to analyze the relationship between *P. pouchetii* abundance and phytoplankton growth and mortality rates.

The results from my study do not support the hypothesis that low grazing mortality rates may be attributed to the presence if *P. antarctica*. Although never the dominant species in terms of overall biomass due to its small cell size, *P. antarctica* was the most abundant species at four stations. If low phytoplankton mortality rates were caused by errors due to the deterrence of grazing by colonial *P. antarctica*, grazing mortality rates should be highest at those experiments with the lowest abundance of colonial *P. antarctica* or at stations where this species was absent; however, this was not found. In fact, non-significant grazing mortality rates were found in experiments both with and without colonial *P. antarctica,* and *P. antarctica* was present in all experiments that yielded significant grazing rates. Additionally, there was no relationship between grazing mortality rates and solitary *P. antarctica* abundance. It is possible that *P. antarctica* may contribute to low grazing rates in the region, but this effect was not observed in this study. It is more likely that these low grazing rates are due to low temperatures; regardless, these low grazing rates suggest that they are not major source of error in ^{14}C-estimates of growth rates.

Loss of fixed ^{14}C in extended incubations

^{14}C-incubations are typically conducted as 24-h incubations in the Ross Sea to accurately measure phytoplankton response over the entire diel cycle while minimizing

bottle effects. However, extending the incubation lengths to 72 h did not affect measured

growth rates. In comparing productivity using ^{14}C- and O_2-methods, Ryther and Vaccaro

(1954) found that although productivity measured by the two methods agreed in 24-h

incubations, the two methods diverged in extended incubations; subsequent studies

attributed this difference to respiration of fixed carbon (Ryther, 1956; Peterson, 1980;

Laws et al., 2000). If respiration by phytoplankton represented a major loss of fixed

carbon in these incubations, measured growth rate should have decreased as incubation

length increased, as respiration initially uses non-labeled carbon (Buckingham et al.,

1975; Hobson et al., 1976; Moigis, 2000). No significant difference in measured growth

rates was observed with extended incubations, indicating that respiration did not

significantly bias growth rate estimates. This is not unexpected, as low temperatures are

expected to yield low respiration rates (Li and Dickie, 1987; Robinson and Williams,

1993; del Giorgio and Duarte, 2002). An additional source of error in long ^{14}C-

incubations might be the ^{14}C-carbon released by grazing. This source of error results in

underestimates of growth rates, and increases with increasing incubation length (Jackson,

1983; Laws et al., 2000; Moigis, 2000). Because grazing mortality rates in the Ross Sea

are low and growth rate estimates in ^{14}C-incubations did not vary with incubation length,

this suggests that grazing did not significantly affect measured growth rates.

Environmental conditions and phytoplankton growth rates in ^{14}C-incubations

Phytoplankton growth rates were variable throughout the region but were low

regardless of the method used to estimate growth, indicating that temperature alone does

not account for the low growth rates seen throughout the region. Phytoplankton growth

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rates in ^{14}C-incubations appeared to be largely influenced by iron concentrations, which were in turn affected by mixed layer depth and POC concentrations (Fig. 13). During the spring and summer in the Ross Sea, one of the primary sources of iron to the euphotic zone is through mixing, as relatively iron-replete water from depth is mixed with iron-deficient surface water (Sedwick et al., 2000). In the spring this effect is likely to play a large role in iron concentrations and phytoplankton growth; the region is characterized by deep mixed layers (ca. 150 m in October; Smith et al., 2000) in which phytoplankton are light-limited (Smith and Gordon, 1997; Smith et al., 2000). Deeper mixing may increase iron input into the euphotic zone, and, if phytoplankton are primarily limited by irradiance, iron concentrations are unlikely to reach limiting levels. If irradiance were limiting growth, the relationship between mixed layer depth and iron concentrations should be relatively strong and positive, with deeper mixed layers correlating to high iron concentrations. Since samples were incubated in shipboard incubators screened to the same irradiance regardless of mixed layer depth, samples from regions with deep mixed layers with high iron concentrations should have yielded relatively high growth rates. Although structural equation modeling indicated that the relationship between iron concentrations and mixed layer depth was positive and there was a positive relationship between mixed layer depth and growth rate, the effect was relatively weak and the relationship was not significant.

As stratification increases, mixed layer depth decreases (mean mixed layers are ca. 20 m in January; Smith et al., 2000) and iron concentrations are primarily influenced by phytoplankton uptake (Sedwick and DiTullio, 1997; Sedwick et al., 2000). With shallow mixed layers, iron input from mixing is not as significant, phytoplankton are

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unlikely to be limited by light, and iron removal is likely to increase (Sedwick and

DiTullio, 1997; Sedwick et al., 2000). This effect would likely be seen by a coupling

between iron concentrations and POC, as increasing biomass removes iron and a strong

positive relationship between iron concentrations and growth rates develop. Structural

equation modeling revealed that the strongest predictor of growth rates was iron

concentrations, followed closely by POC concentrations, indicating that the growth rates

derived from ^{14}C-incubations were likely influenced by low iron concentrations but not

significantly affected by mixed layer depth. While deepening of the mixed layer had a

weak effect on increasing iron concentrations, the negative relationship between POC and

iron concentrations was much stronger, and iron concentrations were more strongly

related to biological than physical conditions, agreeing with previous studies in the spring

(Sedwick et al., 2000). The results of structural equation modeling further confirm the

hypothesis that phytoplankton growth rates are limited by iron concentrations in the late

spring and summer (Smith et al., 2000) due to a decrease in iron inputs as a result of

decreased mixing, reduced ice melt, and increases in iron uptake (Sedwick and DiTullio,

1997; Sedwick et al., 2000).

SUMMARY AND CONCLUSIONS

This study investigated the strengths and weaknesses of two methods for

measuring phytoplankton growth rates and analyzed the relative importance of

temperature, irradiance, and iron concentrations as limiting factors on phytoplankton

growth in the spring in the Ross Sea. The first objective of this study was to assess the

effect of extended incubation length on measured growth rate in dilution experiments and

^{14}C-incubations through the use of time-course experiments. Although the results of

dilution experiments conducted as time-courses were inconclusive, growth rates

measured in ^{14}C-incubations did not change in incubations extended to 72 h. This

suggests that incubation length may be extended in the Ross Sea without significantly

affecting measured growth rate, and that growth rates measured through ^{14}C-incubations

were not significantly affected by the loss of fixed ^{14}C through grazing and respiration.

The second objective of this study was to quantify phytoplankton growth rates

and microzooplankton grazing rates in dilution experiments to test if microzooplankton

grazing rates in the region were low, as found in previous studies, and to compare growth

rates measured using in dilution experiments and in ^{14}C-incubations. Phytoplankton

grazing mortality rates in dilution experiments were low, likely due to low temperatures

in the region, confirming that loss of fixed ^{14}C in ^{14}C-incubations through grazing was

not a significant source of error. These low phytoplankton mortality rates could not be

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directly attributed to the presence of *P. antarctica* in the region, but previous studies have indicated that it may play a role in low microzooplankton grazing rates in the Ross Sea (Caron et al., 2000). Low measured microzooplankton grazing rates indicate that the microbial food web does not play a large role in controlling phytoplankton growth in the region, which has implications for the amount of primary production available for export from the system (Caron et al., 2000; Smith et al., 2007). Although there was no statistically significant difference in growth rates in dilution experiments and ^{14}C-incubations, discrepancies between chlorophyll- and abundance-based growth rates in dilution experiments indicate that chlorophyll-based phytoplankton growth rates from dilution experiments may be inaccurate due to changes in the carbon:chlorophyll ratio of phytoplankton in incubations due to unbalanced growth. Growth rates measured by chlorophyll in dilution experiments should be further assessed by examining cell abundance or POC concentrations over the course of the incubation to determine whether chlorophyll concentrations are reflective of actual growth rates or changing irradiance in the incubations (Schmoker et al., 2013). In low-grazing regions such as the Ross Sea, where physical conditions vary spatially and species such as *P. antarctica* may affect dilution experiments, ^{14}C-incubations are a more appropriate method for measuring growth rates than dilution experiments.

The final objective of this study was to identify factors limiting phytoplankton growth rates measured in ^{14}C-incubations. The results of structural equation modeling indicate that growth rates did not strongly vary with mixed layer depth, which was relatively shallow throughout the sampling region and did not significantly affect iron concentrations. Growth rates were significantly affected by low iron concentrations, most

likely as a result of high biomass and removal of iron. Structural equation modeling of growth rates confirms the hypothesis that phytoplankton growth rates in the austral summer were primarily limited by iron concentrations due to a decrease in iron inputs and increase in iron uptake (Sedwick and DiTullio, 1997; Sedwick et al., 2000).

Future analysis of growth rate measurements in the Ross Sea could benefit from analysis of additional methods for measuring growth rates, such as incorporation of isotopes into protein (DiTullio and Laws, 1983) or dynamics of DNA pools (Carpenter and Chang, 1988). This research was conducted in summer, and further research on phytoplankton growth rates in the Ross Sea could indicate whether the effects of irradiance on carbon:chlorophyll significantly affects chlorophyll-based measurements in spring. A similar study conducted in the spring is likely to yield different results regarding the relative importance of irradiance and iron as limiting factors on phytoplankton growth and could further support the low phytoplankton grazing rates found in this and previous studies (Caron et al., 2000). Future research on phytoplankton growth rates in the Ross Sea could provide a more comprehensive view of how limitations on phytoplankton growth vary seasonally and could further indicate the relative suitability of the various methods to measure growth rates in the region.

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Table 1. Phytoplankton growth rates (d^{-1}) in the Ross Sea determined by different methods.

Location	Season	Growth Rate (d^{-1})	Measurement method	Reference
Ross Sea	Summer 1983	0.07-0.33	^{15}N-uptake	Nelson & Smith, 1986
Ross Sea	Summer 1994/1995	0.09-0.49	Change in nitrate concentration	Smith et al. 1998
Ross Sea	Summer 1994/1995	0.14-0.26	Change in POC concentration	Smith et al. 1998
Ross Sea	Summer 1994/1995	0.14-0.27	Change in PON concentration	Smith et al. 1998
Ross Sea	Summer 1994/1995	(-0.02)-0.13	Change in chlorophyll concentration	Smith et al. 1998
Ross Sea	Summer 1994/1995	0.26-0.35	^{14}C-uptake	Smith et al. 1998
Ross Sea	Spring 1994	0.04-1.02	^{14}C-uptake	Smith et al. 1999
Ross Sea	Spring 1994	0.02-0.23	^{15}N-uptake	Smith et al. 1999
Ross Sea	Spring 1994	0.08-0.12	^{32}Si-uptake	Smith et al. 1999
Ross Sea	Summer 1995/1996	0.02-0.41	^{14}C-uptake	Smith et al. 1999
Ross Sea	Summer 1995/1996	0.01-0.17	^{15}N-uptake	Smith et al. 1999
Ross Sea	Summer 1995/1996	0.03-0.15	^{32}Si-uptake	Smith et al. 1999
Ross Sea	Spring 1994	0.02-0.18	Change in POC concentration	Smith et al. 1999
Ross Sea	Spring 1994	0.01-0.13	Change in PON concentration	Smith et al. 1999
Ross Sea	Spring 1994	0.03-0.53	Change in chlorophyll concentration	Smith et al. 1999
Ross Sea	Spring 1996/1997	0.11-0.47	^{14}C-uptake	Smith et al. 2000
Ross Sea	Summer 1997	0.059	^{14}C-uptake	Smith et al. 2000

Table 2. Phytoplankton cell volume and carbon content (±S.E.) used to calculate biomass from cell abundance data. *P. antarctica = Phaeocystis antarctica.*

Species	Cells Sized	Average Length (μm)	Average Width (μm)	Shape	Average Volume (μm^3)	Average Carbon Content (pg C)
P. antarctica (solitary)	50	3.4 ± 0.8	NA	Sphere	21.0 ± 2.5	1.0 ± 0.1
P. antarctica (colonial)	130	4.7 ± 0.9	NA	Sphere	55.1 ± 2.3	1.6 ± 0.0
Cylindrotheca closterium	100	71.5 ± 0.9	2.5 ± 0.0	Prolate Spheroid	234 ± 7.6	28.1 ± 0.7
Fragilariopsis spp.	100	28.9 ± 0.6	4.6 ± 0.0	Rectangle	621 ± 42.3	59.0 ± 3.0
Dactyliosolen spp.	100	37.9 ± 0.9	11.5 ± 0.7	Cylinder	3940 ± 608.2	240 ± 27.7
Nitzschia longissima	100	1534 ± 2.6	2.5 ± 0.0	Cylinder	740 ± 27.5	67.4 ± 1.9
Chaetoceros spp.	50	19.9 ± 0.9	13.0 ± 0.4	Prolate Spheroid	1770 ± 206	131 ± 11.4
Pseudo-nitzschia spp.	100	92.7 ± 1.2	5.6 ± 0.2	Prolate Spheroid	1520 ± 125	116 ± 7.2
Dictyocha speculum	50	24.5 ±0.2	NA	Sphere	7680 ± 179	614 ± 14.4
Dinoflagellate (>20 μm)	100	35 ± 0.4	24.4 ± 0.3	Prolate Spheroid	10,900 ± 825.9	261 ± 17.7
Dinoflagellate (<20 μm)	75	19.7 ± 0.9	12.5 ± 0.6	Prolate Spheroid	1600 ± 127	1370 ± 88.9

Table 3. Phytoplankton grazing mortality and growth rates from dilution experiments in the Ross Sea from 8 January 2012 to 28 January 2012 (Fig. 1). Date indicates the date each station, indicated by station number and a brief description of the station, was sampled. Initial biomass is based on fluorometrically-derived chlorophyll a concentrations (μg L^{-1}) at the time of sampling. Grazing mortality rate (g; d^{-1}) is indicated for stations with mortality rates that significantly differed between treatments (p>0.05; NS=not significant). Growth rates both by chlorophyll and cell abundances are included. Intrinsic growth rates are indicated for stations with mortality rates that differed significantly between treatments (NA=not available).

Date	Station Number	Station Description	Initial Biomass (µg Chl a L⁻¹)	Incubation Length (h)	g (d⁻¹)	Chlorophyll-Based Net Growth Rate (d⁻¹)	Abundance-Based Net Growth Rate (d⁻¹)	Chlorophyll-Based Intrinsic Growth Rate (d⁻¹)	Abundance-Based Intrinsic Growth Rate (d⁻¹)
01.08.12	3	Outside Ice	1.08	72	0.18	-0.02	0.16	0.16	0.34
01.09.12	4	Eddy Center	9.45	24	NS	-0.29	-0.03	NA	NA
				48	NS	-0.08	0.27	NA	NA
				72	NS	0.00	0.15	NA	NA
01.14.12	9	Low Biomass	1.65	72	0.30	0.26	0.13	0.56	0.43
01.17.12	19	Frontal Region	1.71	72	NS	0.47	0.12	NA	NA
01.17.12	22	Low Biomass	0.93	24	NS	0.23	-0.14	NA	NA
				48	-0.30	0.51	-0.10	0.21	-0.40
				72	-0.20	0.47	0.06	0.27	-0.14
01.21.12	37	Ross Bank	4.66	72	NS	0.05	0.16	NA	NA
01.22.12	52	Ross Bank	2.96	72	NS	0.12	0.15	NA	NA
01.25.12	60	Ross Ice Shelf	5.95	72	NS	-0.09	0.05	NA	NA
01.25.12	61	Ross Ice Shelf	7.14	72	NS	-0.12	0.20	NA	NA
01.28.12	79	Joides Trough	0.22	24	-0.32	0.05	-0.31	-0.29	-0.63
				48	NS	0.11	0.04	NA	NA
				72	NS	0.10	0.08	NA	NA
01.28.12	80	Joides Trough	0.33	72	0.09	-0.01	0.10	0.08	0.19

Table 4. Growth rates (d^{-1}) in 24-, 48-, and 72-h ^{14}C-incubations. Temperature (°C) and mixed layer depth (m) based on CTD profile and bottle data. St. = station number (Fig. 1); POC = particulate organic carbon.

St.	Description	T (°C)	MLD (m)	POC (μM)	Growth Rate (d^{-1}) 24-h	48-h	72-h
3	Outside Ice	-0.79	33	0.94	0.85	0.59	0.49
4	Eddy 1 Center	-0.17	41	18.4	0.27	0.21	0.27
7	Ice Edge	-0.74	14	20.05	0.18	0.18	0.17
9	Low Biomass	0.95	20	6.79	0.05	0.22	0.30
14	Eddy 3 Center	2.11	11	40.25	0.09	0.09	0.07
19	Frontal Region	1.14	18	11.56	0.19	0.22	0.12
22	Low Biomass	1.37	12	12.65	0.10	0.13	0.13
37	Ross Bank	0.13	19	31.72	0.11	0.10	0.10
52	Ross Bank	-0.25	39	20.85	0.11	0.10	0.10
56	Ross Ice Shelf	-0.09	18	25.76	0.13	0.14	0.12
57	Ross Ice Shelf	-0.35	28	47.3	0.06	0.06	0.06
58	Ross Ice Shelf	-0.15	40	53.81	0.10	0.08	0.03
59	Ross Ice Shelf	-0.74	54	30.59	0.12	0.07	0.08
60	Ross Ice Shelf	-0.69	97	24.07	0.19	0.16	0.12
61	Ross Ice Shelf	-0.42	39	43.05	0.09	0.08	0.07
62	Ross Ice Shelf	-0.55	43	9.06	0.43	0.27	0.29
75	Ross Bank	-0.68	47	19.82	0.15	0.10	0.08
76	Ross Bank	-0.28	63	20.05	0.08	0.08	0.06
79	Joides Trough	0.10	20	7.26	0.04	0.04	0.09
80	Joides Trough	-0.11	49	6.17	0.06	0.06	0.13
81	Joides Trough	-1.00	10	10.54	0.10	0.08	0.09
82	Joides Trough	-0.26	28	2.34	0.18	0.22	0.22
83	Joides Trough	0.01	41	1.8	0.14	0.13	0.16
84	Joides Trough	-0.37	13	6.95	0.18	0.19	0.22
85	Joides Trough	-0.45	18	5.86	0.10	0.12	0.11
86	Joides Trough	-0.53	12	7.65	0.07	0.19	0.21
88	Joides Trough	0.25	38	13.59	0.08	0.08	0.07
89	Joides Trough	0.23	29	13.12	0.05	0.04	0.04
92	Joides Trough	0.10	27	34.27	0.08	0.08	0.10
93	Low Biomass	-0.44	16	13.2	0.11	0.11	0.12
94	High Biomass	-0.07	10	33.06	0.17	0.21	0.20
95	Ice Edge	-1.12	10	35.76	0.16	0.16	0.18
96	Eddy Center	0.51	17	54.94	0.12	0.09	0.15
97	East Eddy Center	0.16	11	52.96	0.06	0.07	0.07
98	South Eddy Center	0.36	10	53.53	0.05	0.06	0.08
101	Franklin Island	-1.31	10	22.88	0.11	0.10	0.12
102	169 °E	0.05	10	36.51	0.12	0.13	
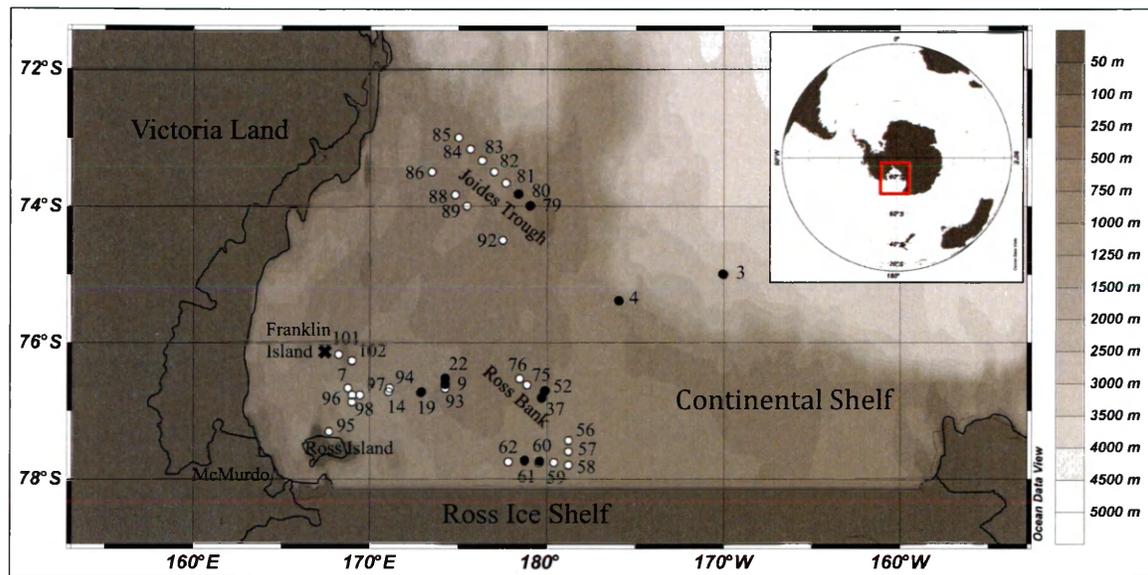
Figure 1. Map of stations in the Ross Sea sampled from 8 January 2012 to 2 February

2012 on NBP12-01. Eleven stations were sampled for both dilution experiments and [14]C-

incubations (black points) and 26 stations were sampled for ^{14}C-incubations (white

points). Contours indicate water depth (m). Temperatures ranged from (-1.31)-2.11°C,

mixed layer depth ranged from 10-97 m, particulate organic carbon concentrations ranged

from 0.9-54.9 µM, and chlorophyll *a* concentrations ranged from 0.22-10.5 µg L^{-1} at

stations sampled (Appendix 1).
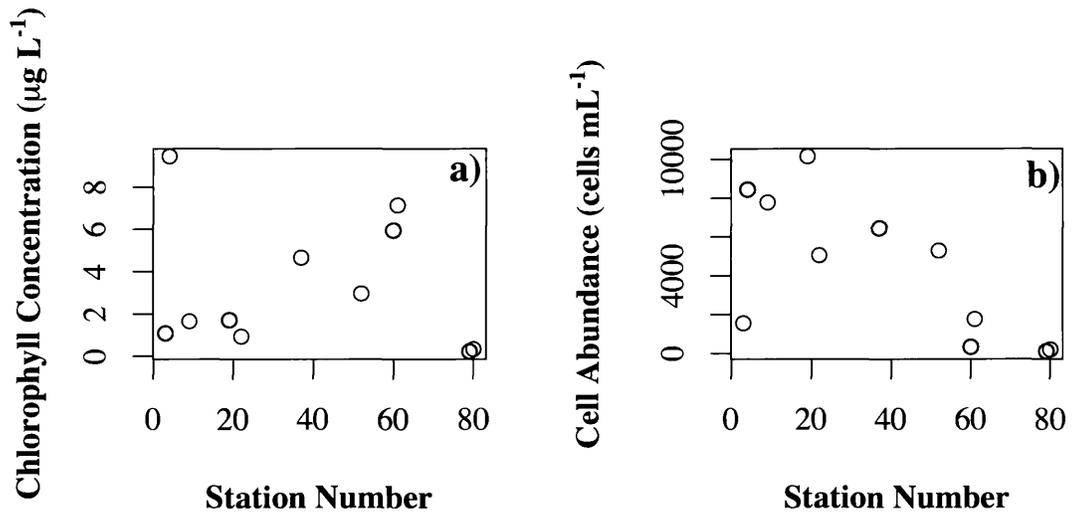
Figure 2. Phytoplankton standing stock at 10 m at stations sampled for dilution experiments (Table 3; Fig. 1) as a) initial chlorophyll (μg Chl a L^{-1}) and b) cell abundance (cells mL^{-1}).
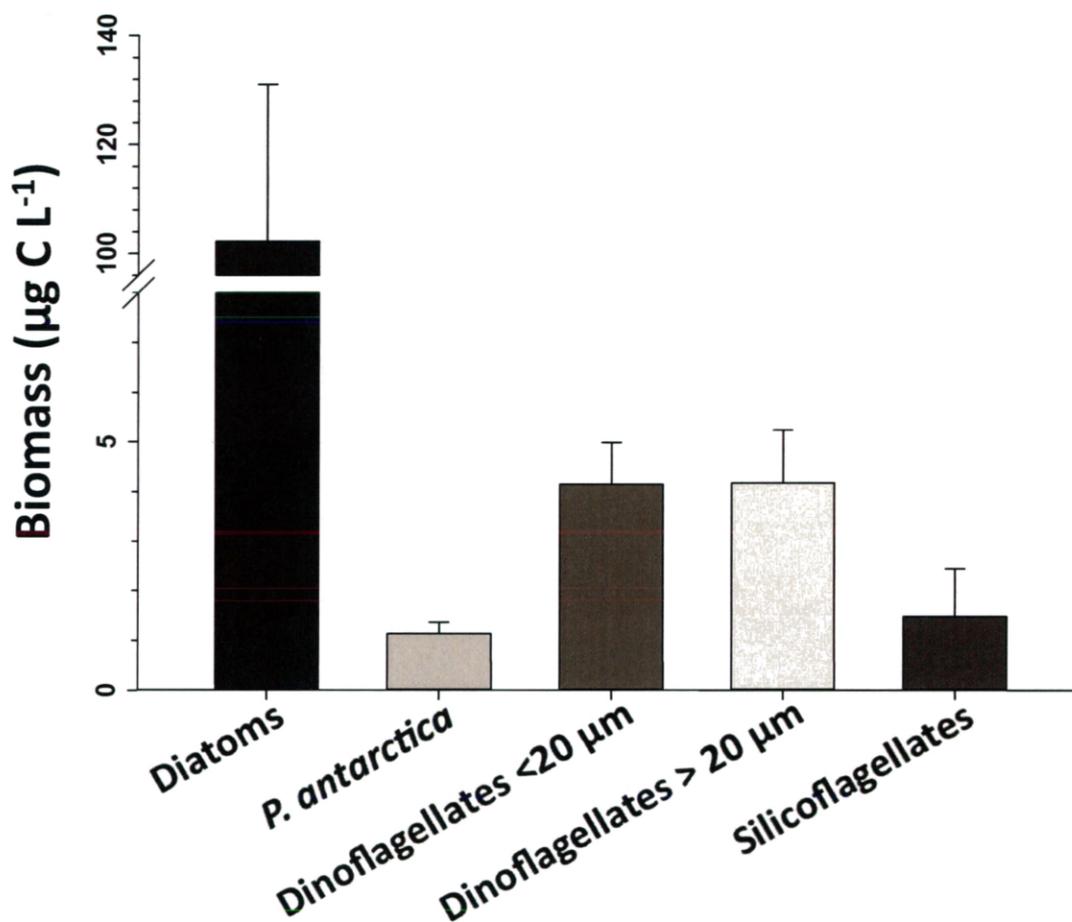
Figure 3. Mean initial biomass (μg C L^{-1}) of phytoplankton in dilution experiments (n=11) derived from cell length/width measurements and conversion into carbon units using literature volume to carbon conversions. Error bars are standard errors.

Figure 4. Initial phytoplankton composition in dilution experiments. Percentage of total abundance (% of total cells mL^{-1}) based on average abundance in cell counts. *Cylindrotheca closterium, Fraglariopsis spp., Dactyliosolen spp., Nitzschia longissima, Chatoceros spp., and Pseudo-nitzschia spp.* are diatoms. The prymnesiophyte *P. antarctica (Phaecosystis antarctica)* was evaluated based on whether cells were present in solitary or colonial form. Dinoflagellates were classified based on size. *Dictyocha speculum* is a silicoflagellate.

65
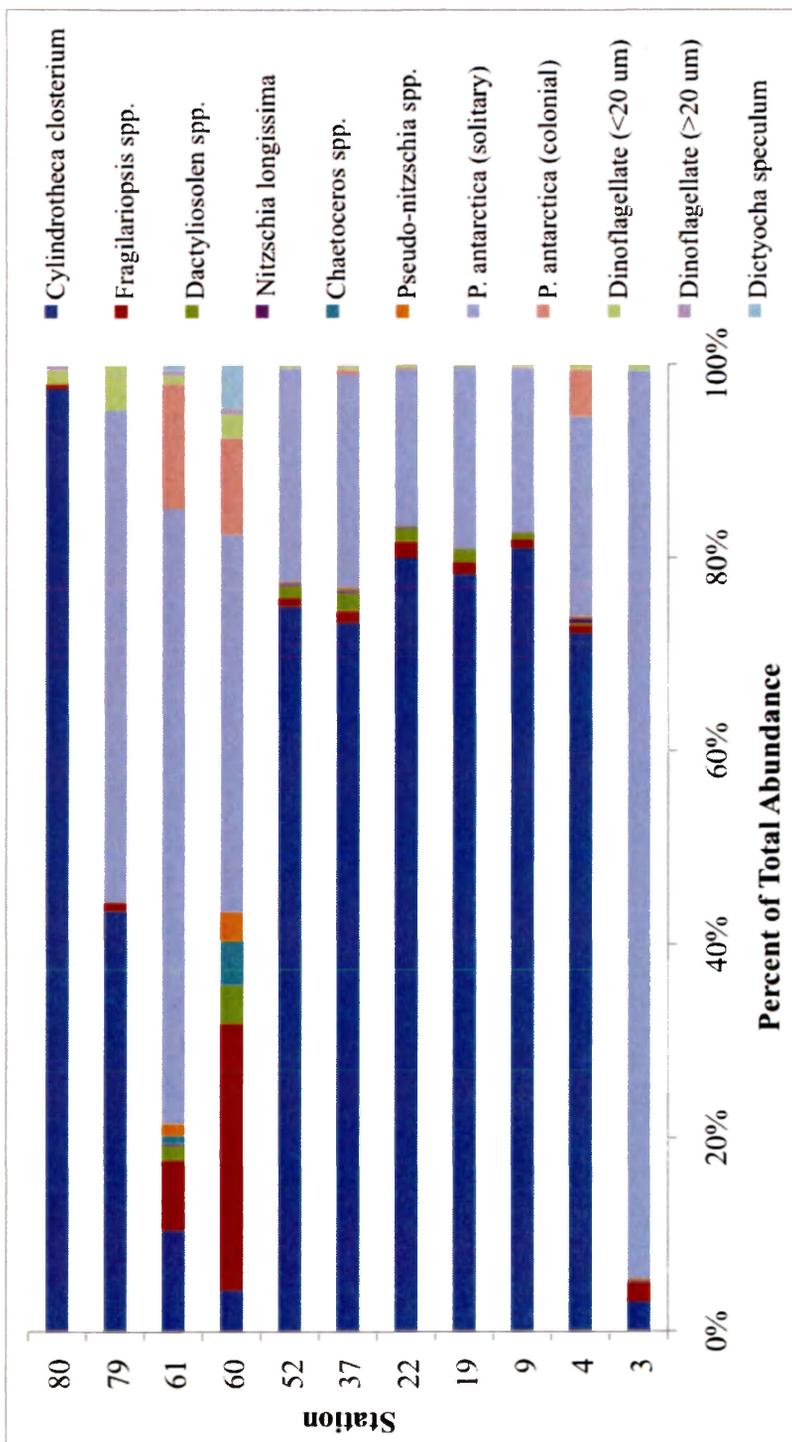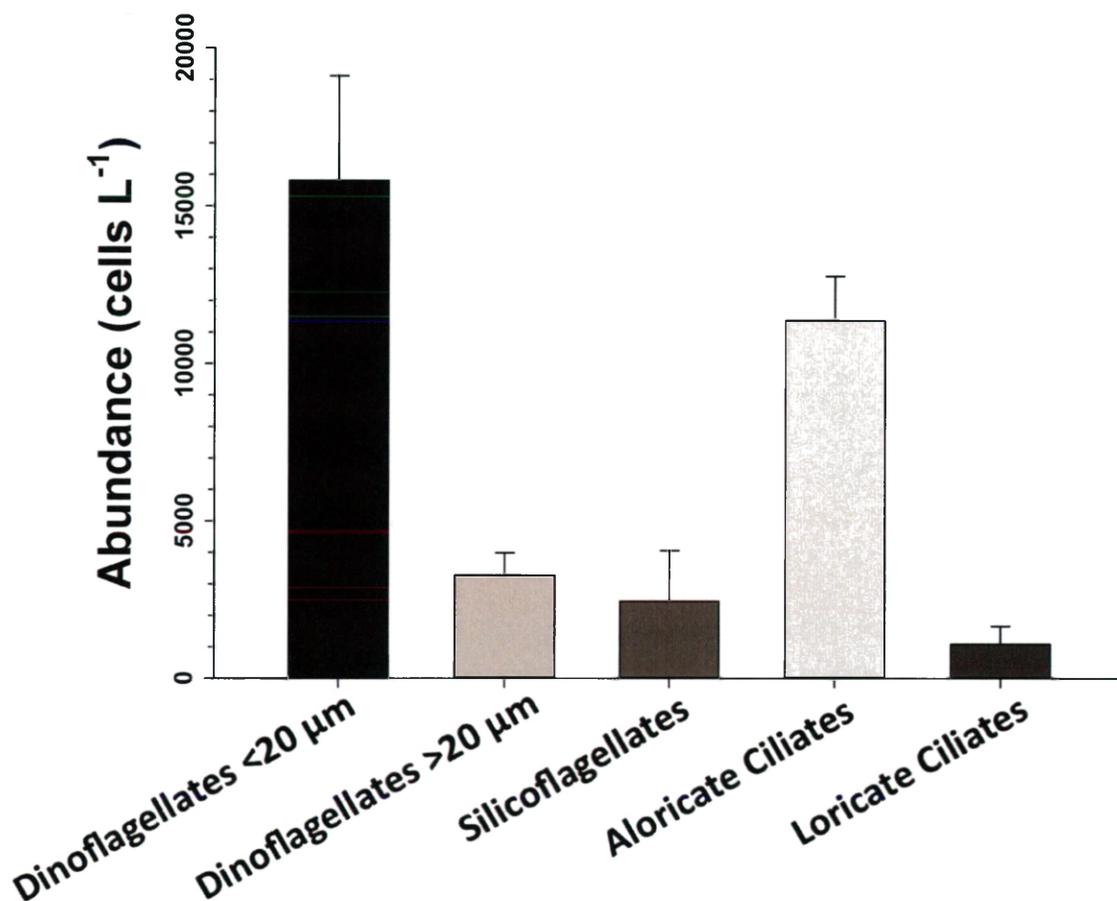
Figure 5. Mean initial abundance (cells L^{-1}) of plankton with demonstrated heterotrophic behavior in dilution experiments (n=11). Error bars are standard errors. Dinoflagellate and silicoflagellate abundance includes all individuals of that functional group as individuals were not evaluated based on observed heterotrophy or autotrophy.

Figure 6. Phytoplankton grazing mortality rates (d^{-1}) in dilution experiments as a function of net growth rates (d^{-1}) based on a) chlorophyll and b) cell abundances and at varying initial c) abundance (cells mL^{-1}) and d) biomass ($\mu g\ C\ mL^{-1}$) of colonial *P. antarctica (Phaeocystis antarctica)*. All non-significant phytoplankton grazing mortality rates are set to zero.
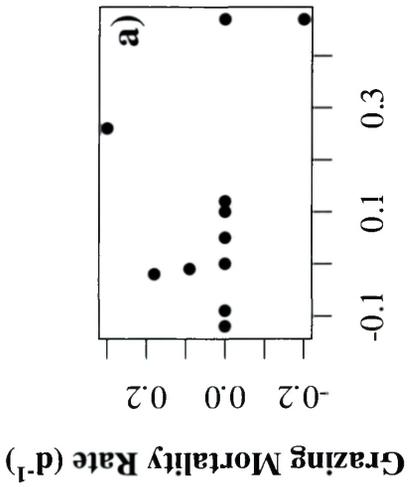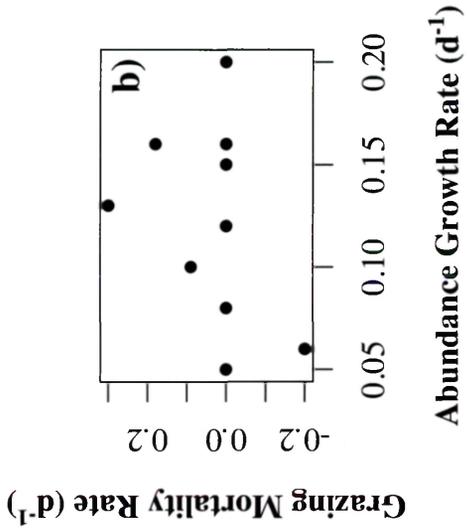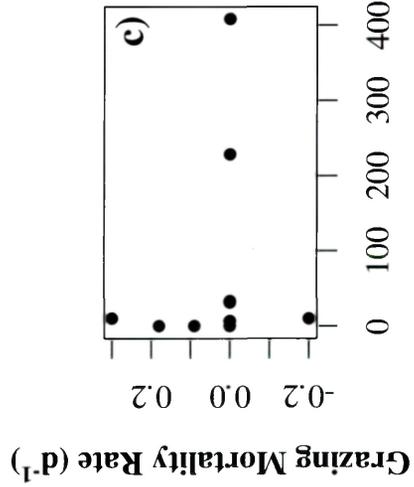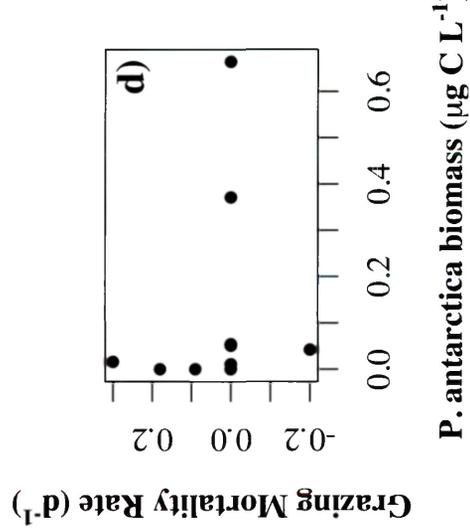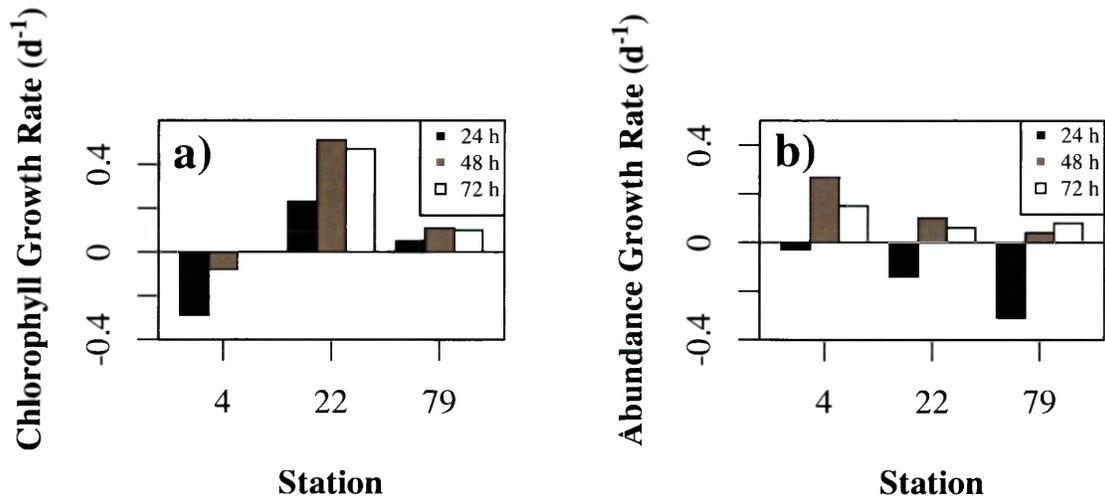
Figure 7. Net growth rates (d^{-1}) of phytoplankton based on a) changes in chlorophyll and b) changes in cell abundances in time-course dilution experiments.
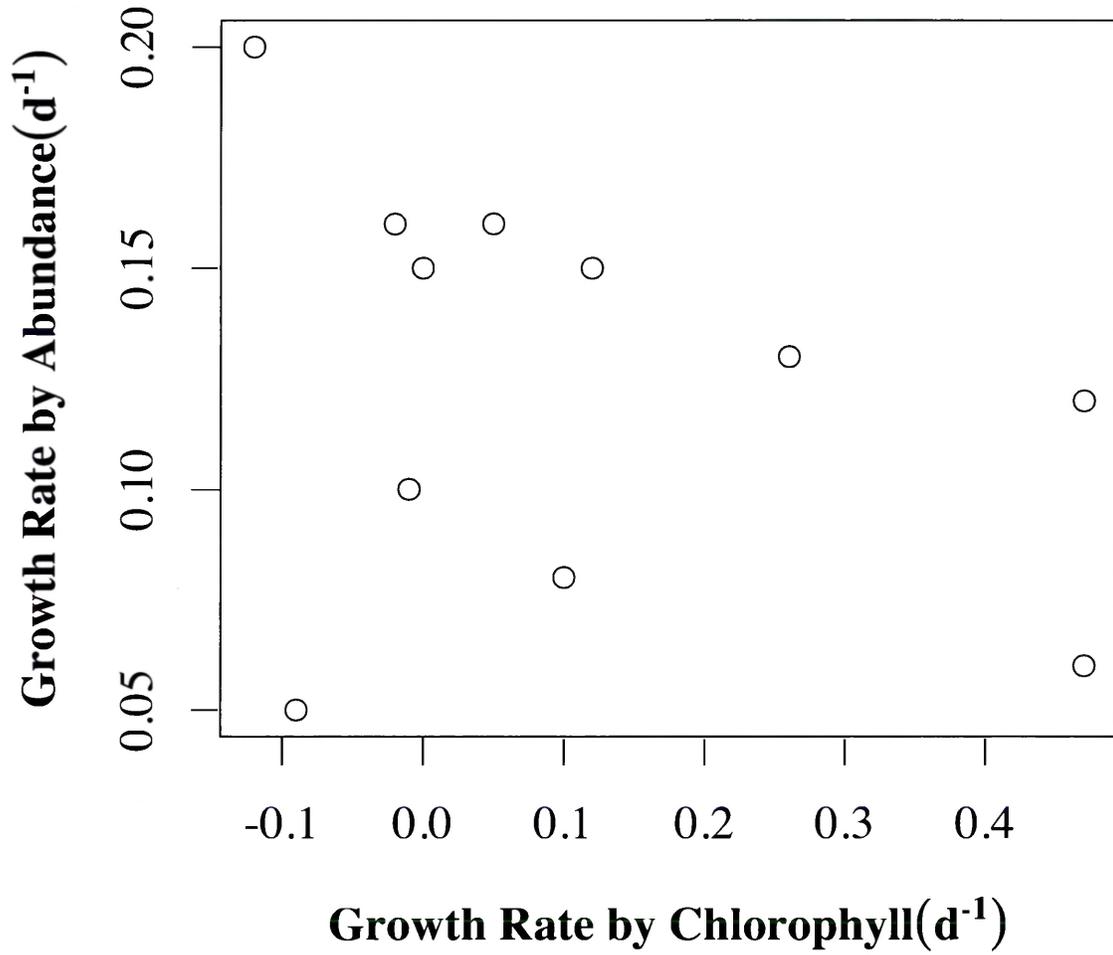
Figure 8. Relationship between net growth rates (d[-1]) based on chlorophyll and cell abundances in dilution experiments.
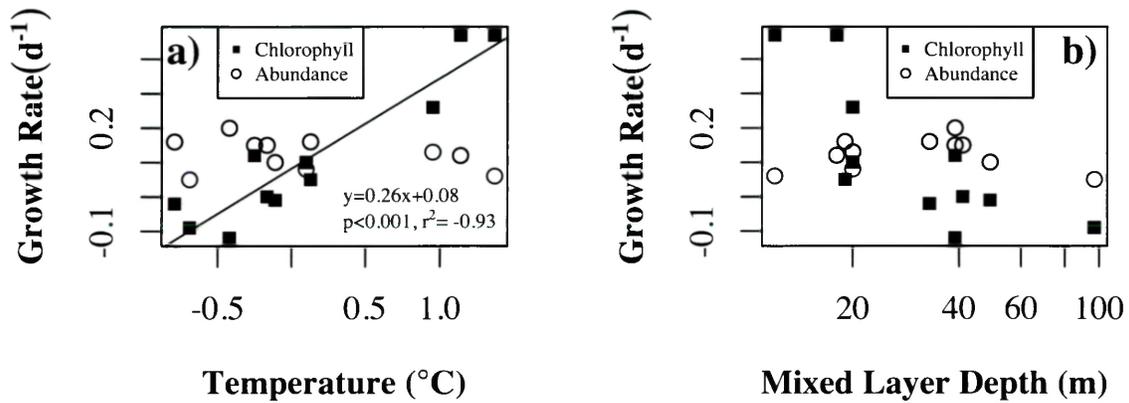
Figure 9. Relationship between a) temperature (°C) and b) mixed layer depth (m) vs. net growth rate (d^{-1}) in dilution experiments based on chlorophyll (black squares) and cell abundances (white circles). Line in (a) indicates a significant correlation between chlorophyll-based net growth rate and temperature. Spearman's rank correlation indicated a significant relationship between mixed layer depth and chlorophyll-based net growth rate and mixed layer depth (P=-0.78; p=0.005) in (b).

Figure 10. Relationship between carbon:chlorophyll ratios based on microscopy and environmental conditions. Initial carbon:chlorophyll ratios increased with a) increasing temperature (°C) and b) decreasing mixed layer depth (m). Percent change in carbon:chlorophyll ratio increased with a) decreasing temperature and b) increasing mixed layer depth. Decreasing initial carbon:chlorophyll ratios and increasing percent change in carbon:chlorophyll ratios with increasing mixed layer depth based on Spearman's rank correlations (P=-0.69; p=0.01 and P=0.80; p=0.01, respectively).
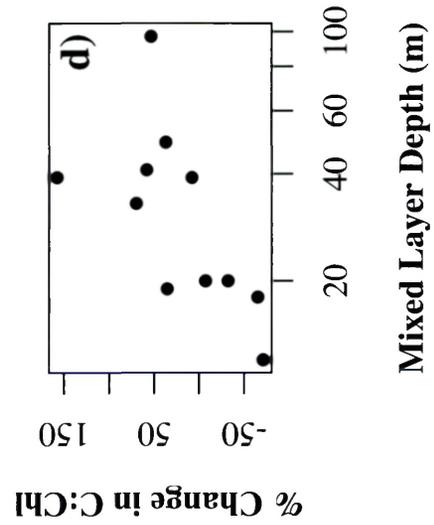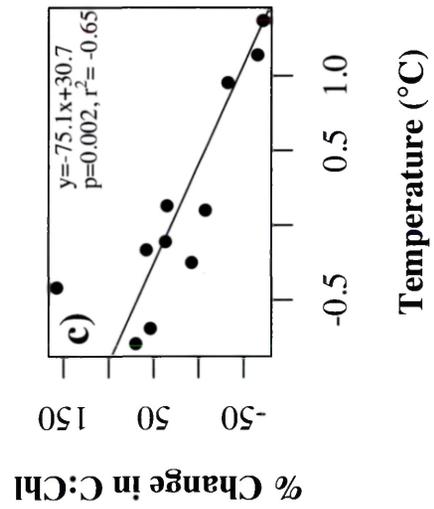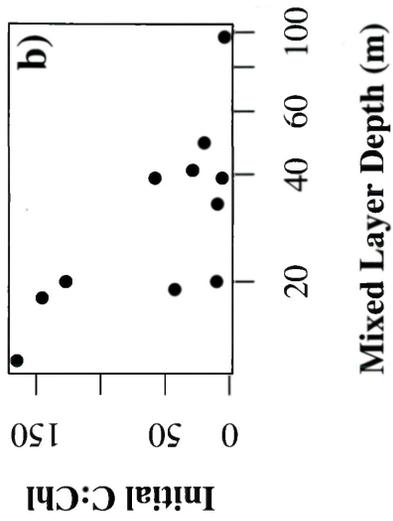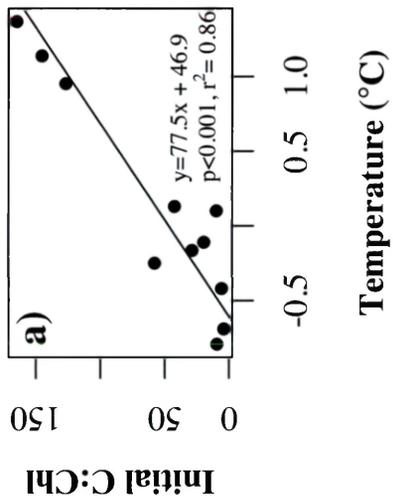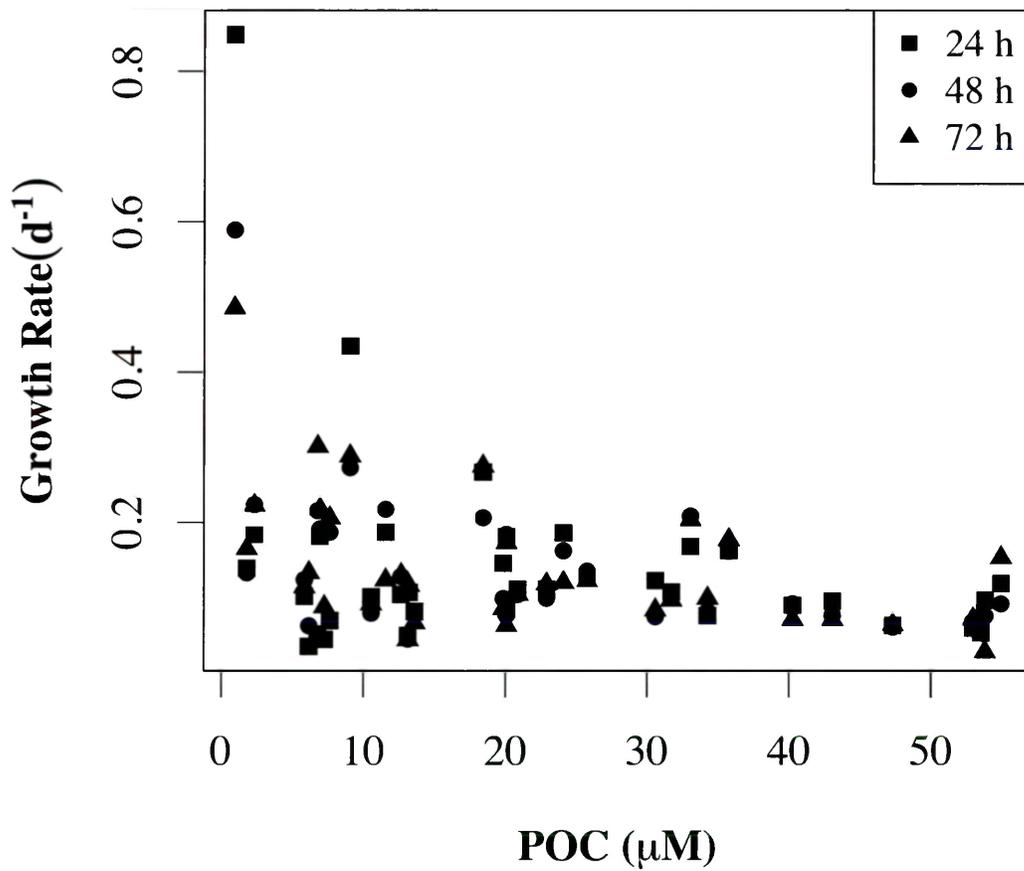
Figure 11. Phytoplankton growth rates (d^{-1}) from ^{14}C-incubations in 24- (square), 48- (circle), and 72-h (triangle) incubations. Growth rates decreased with increasing initial POC concentrations (μM; r^2=0.22). Statistical analyses run using log-transformed POC concentrations and growth rates.
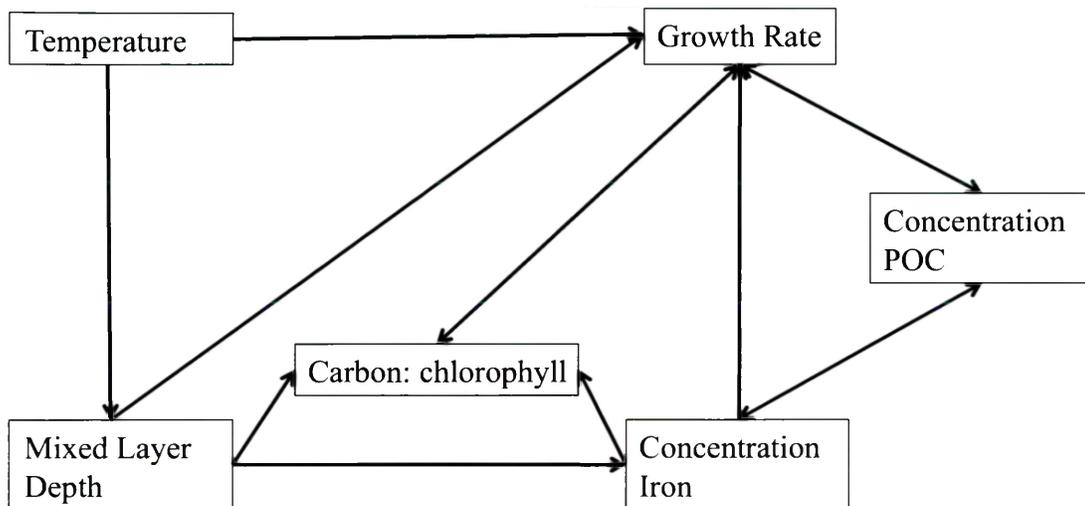
Figure 12. Model design for structural equation modeling on stations sampled for ^{14}C-incubations and environmental data at 10 m. Double-headed arrows indicate intercorrelation between variables and single directional arrows indicate paths of prediction.

Figure 13. Results of Structural equation modeling on stations sampled for ^{14}C-incubations. Weight of the line indicates the strength of the relationship between variables; bold lines indicate strong relationships and dashed lines indicate weak relationships between variables based on model results. Coefficients on arrows pointing to center of boxes indicate amount of variance in that parameter explained by the model (r^2 value for the parameter). Coefficients on unidirectional arrows are the prediction coefficient for the relationship indicated; they indicate the change (in standard deviations) expected in the response variable following a 1.0 standard deviation change in the predictor variable. Coefficients on double-headed arrows indicate the strength of the intercorrelation between variables.
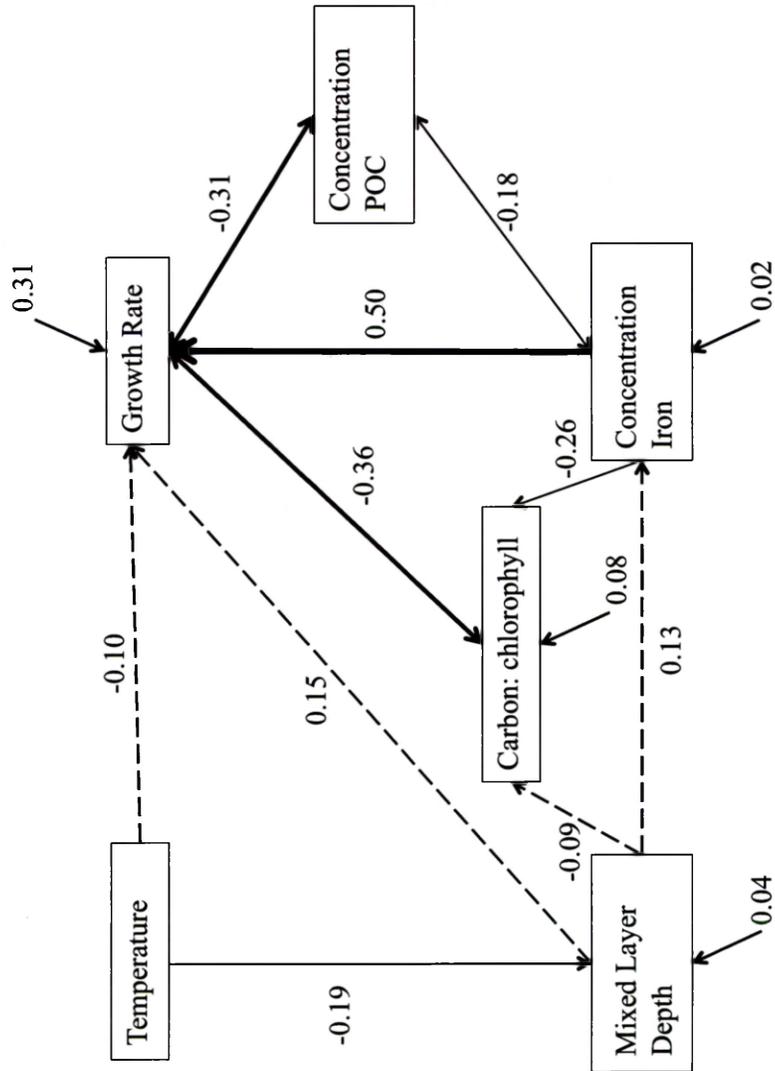
APPENDIX

Appendix 1. Physical and biological data included in structural equation model analysis.

Stn= station number (Fig. 1); Temp= water temp at 10 m; MLD= mixed layer depth

based on 0.01 unit change in σ_t from σ_t at 10 m; Fe= iron concentrations at 10 m; POC=

particulate organic carbon at 10 m; C:Chl= carbon:chlorophyll ratio based on chlorophyll

a concentrations determined flurometrically and particulate organic carbon at 10 m; GR=

growth rates in 24-h ^{14}C-incubations. A dash (-) indicates data not available. Iron

concentrations courtesy of Sedwick et al. (in prep).

Stn	Temp (°C)	MLD (m)	Fe (nM)	POC (µM)	C:Chl	GR (d^{-1})
3	-0.79	33	0.285	0.94	10.4	0.85
4	-0.17	41	0.422	18.4	23.4	0.27
7	-0.74	14	0.092	20.1	52.3	0.18
9	-0.95	20	0.068	6.79	49.4	0.05
14	2.11	11	0.049	40.3	45.9	0.09
19	1.14	18	0.061	11.6	81.1	0.19
22	1.37	12	-	12.7	163	0.10
37	0.13	19	-	31.7	81.7	0.11
52	-0.25	39	-	20.9	84.6	0.11
56	-0.09	18	0.075	25.8	36.0	0.13
57	-0.35	28	-	47.3	79.1	0.06
58	-0.15	40	0.071	53.8	95.0	0.10
59	-0.74	54	-	30.6	55.3	0.12
60	-0.69	97	0.054	24.1	48.6	0.19
61	-0.42	39	-	43.1	69.7	0.09
62	-0.55	43	0.079	9.06	24.4	0.43
75	-0.68	47	-	19.8	175	0.15
76	-0.28	63	-	20.1	254	0.08
79	0.10	20	0.079	7.26	393	0.04
80	-0.11	49	-	6.17	222	0.06
81	-1.00	10	-	10.5	207	0.10
82	-0.26	28	0.073	2.34	56.2	0.18

Appendix 1. continued

Stn	Temp (°C)	MLD (m)	Fe (nM)	POC (µM)	C:Chl	GR (d^{-1})
83	0.01	41	-	1.80	39.1	0.14
84	-0.37	13	-	6.95	150	0.18
85	-0.45	18	0.074	5.86	111	0.10
86	-0.53	12	0.033	7.65	107	0.07
88	0.25	38	-	13.6	305	0.08
89	0.23	29	0.048	13.1	349	0.05
92	0.10	27	0.056	34.3	79.8	0.08
93	-0.44	16	0.054	13.2	92.4	0.11
94	-0.07	10	0.049	33.1	58.0	0.17
95	-1.12	10	0.068	35.8	110	0.16
96	0.51	17	0.064	54.9	90.7	0.12
97	0.16	11	-	53.0	90.6	0.06
98	0.36	10	-	53.5	80.0	0.05
101	-1.31	10	0.198	22.9	58.2	0.11
102	0.05	10	0.086	36.5	113	0.12

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